

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**203629Orig1s000**

**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**

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CDER stamp date: 10/15/2013, 03/12/2014 and 07/11/2014  
Product: Neostigmine Methylsulfate Injection  
Indication: Reversal agent to the neuromuscular blocking effects of non-depolarizing muscle relaxants  
Applicant: Fresenius Kabi  
Review Division: Division of Anesthesia, Analgesia, and Addiction Products  
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# 1 Executive Summary

## 1.1 Introduction

A complete response letter was sent to the Applicant for the original NDA submission as a result of a failed facility inspection (01/29/2013). There were no nonclinical issues that prevented the approval of this NDA. However, in the complete response letter, the applicant was informed that if the NDA were to have been approved, they would be required to conduct a total of seven studies. Those studies included two genotoxicity studies on neostigmine methylsulfate (in vitro chromosomal aberration assay in mammalian cells and in vivo chromosomal damage assay), a battery of reproductive and developmental toxicology studies (fertility and early embryonic developmental study in rats, embryonic developmental study in rats and rabbits; and pre- and post-natal developmental study in rats), and an adequate extractable/leachable safety assessment for the (b) (4) gray (b) (4) rubber stopper used in the container closure system. In the current submissions, the Applicant submitted six of the seven nonclinical studies that would have been recommended as post-marketing requirements and update labeling (7/11/2014). The nonclinical studies address six of the seven original recommended PMRs, with the exception of the extractable/leachable study. This nonclinical review covers these genotoxicity and reproductive and developmental toxicology studies and nonclinical parts of the drug product label.

## 1.2 Brief Discussion of Nonclinical Findings

### Genotoxicity:

The two submitted genotoxicity studies, an in vitro chromo aberration assay in human peripheral blood lymphocytes and an in vivo rat bone marrow micronucleus assay, were reviewed and the results suggest that neostigmine did not demonstrate genotoxic potential under the conditions of the studies.

Based on these negative findings and negative finding in the Ames test that was previously reviewed (9/18/2013), neostigmine methylsulfate is not mutagenic or clastogenic.

### Reproductive and Developmental Toxicology:

A standard battery of reproductive and developmental toxicology studies were submitted. All studies used the intravenous route to administer neostigmine methylsulfate as a daily bolus dose. The dose levels were 0, 10, 25, and 50 mcg/kg in rats; 0, 10, 25, and 40 mcg/kg in rabbits. The high dose tested, 50 mcg/kg in rats, 40 mcg/kg in rabbits were considered acceptable based on the treatment-related clinical finding of tremor/twitch following dose administration. There were no treatment-related adverse findings in the fertility and early embryonic development study in rats, embryonic fetal development study in rats and rabbits, or the pre- and post-natal development study in rats. NOAELs for reproductive toxicity were defined as 50 mcg/kg for the rat studies and 40 mcg/kg for the rabbit study. These NOAELs represent human

equivalent doses (on a mg/m<sup>2</sup> basis) of 8.06 mcg/kg (rat data) and 12.9 mcg/kg (rabbit data).

Compared to the maximum recommended human dose of 5 mg (83 mcg/kg for a 60 kg human body), these animal NOAELs are 6.5-10.3 times lower than the MRHD based on mg/m<sup>2</sup> basis. Therefore, these negative findings in reproductive toxicology studies are of limited clinical relevance.

### 1.3 Recommendations

There are no approvability issues. The currently reported genotoxicity and reproductive and developmental toxicology data should be incorporated into the labeling.

The previously recommended leachable/extractable assessment should be required to be completed by the Sponsor as a Postmarketing Requirement.

#### 1.3.3 Labeling

The labeling recommendations below have not been discussed with the entire review team or the Applicant. The reader is referred to the action letter for final drug product labeling.

The Sponsor proposed language	This P/T Reviewer suggested language	Comment
<p><b>8.1 Pregnancy</b></p> <p>(b) (4)</p> <p>(b) (4) There are no adequate or well-controlled studies of Neostigmine Methylsulfate Injection in pregnant women. It is not known whether Neostigmine Methylsulfate Injection can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity.</p> <p>(b) (4)</p>	<p><b>8.1 Pregnancy</b></p> <p>(b) (4)</p> <p>(b) (4) <del>There are no adequate or well-controlled studies of Neostigmine Methylsulfate Injection in pregnant women. It is not known whether Neostigmine Methylsulfate Injection can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity.</del></p> <p>(b) (4)</p> <p>Risk Summary Adequate and well-controlled studies with Neostigmine Methylsulfate Injection have not been conducted in pregnant women. Although animal studies showed no treatment related effects on embryo-fetal development or pre- and post-natal development, the</p>	<p>The pregnancy category may be removed as per the pregnancy Labeling and Lactation (PLLR) Final Rule. A risk summary statement replaces the category.</p>

	<p>animal doses evaluated corresponded to doses lower than the maximum recommended human dose (MRHD) of 5 mg/60 kg person on a mg/m<sup>2</sup> basis. Neostigmine Methylsulfate Injection should only be used during pregnancy if clearly needed.</p> <p><b>Animal Data</b> In embryofetal development studies, rats and rabbits were administered neostigmine methylsulfate at human equivalent doses (HED, on a mg/m<sup>2</sup> basis) of 1.6, 4.0 and 8.1 mcg/kg/day 3.2, 8.1, and 13.0 mcg/kg/day, respectively, during the period of organogenesis (Gestation Days 6 through 17 for rats and Gestation Days 6 through 18 for rabbits). There was no evidence for a teratogenic effect in rats and rabbits up to HED 8.1 and 13.0 mcg/kg/day, which are approximately 0.097-times and 0.16-times the MRHD of 5 mg/60 kg, respectively.</p> <p>In a pre- and postnatal development study in rats, neostigmine methylsulfate was administered to pregnant female rats at human equivalent doses (HED) of 1.6, 4.0 and 8.1 mcg/kg/day from Day 6 of gestation through Day 20 of lactation, with weaning on Day 21. No adverse effects on physical development, behavior, learning ability, or fertility in the offspring occurred at HED doses up 8.1 mcg/kg/day which is 0.097-times the MRHD of 5 mg/60 kg on a mg/m<sup>2</sup> basis.</p> <p>In these studies, no significant maternal toxicities were observed other than tremors/twitching after administration.</p>	
<p>(b) (4)</p>	<p><b>13 NONCLINICAL TOXICOLOGY</b> <b>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</b> <i>Carcinogenesis:</i> Long-term animal studies have not been performed to evaluate the carcinogenic potential of Neostigmine Methylsulfate Injection.</p> <p><i>Mutagenesis:</i> Neostigmine Methylsulfate Injection was not mutagenic or clastogenic when evaluated in an in vitro bacterial reverse mutation (Ames test), an in vitro</p>	

(b) (4)	<p>chromosomal aberration assay in human peripheral blood lymphocytes, and an in vivo rat bone marrow micronucleus assay.</p> <p><i>Impairment of Fertility:</i> In a fertility and early embryonic development study in rats, male rats were treated for 28-days prior to mating and female rats were treated for 14 days prior to mating through Gestation Day 7 with intravenous administration of Neostigmine Methylsulfate at human equivalent doses of 1.6, 4.0 and 8.1 mcg/kg/day. No adverse effects on fertility and early embryonic development occurred at any dose (up to 0.097-times the MRHD of 5 mg/60 kg on a mg/m<sup>2</sup> basis).</p>	<p>These data are included in Section 8.</p>
	(b) (4)	

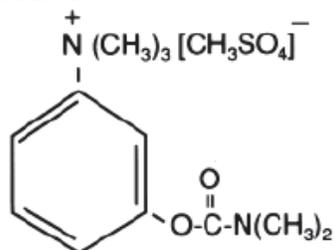
## 2 Drug Information

### 2.1 Drug

CAS Registry Number	51-60-5
Generic Name	Neostigmine methylsulfate
Code Name	N/A
Chemical Name	(m-hydroxyphenyl) trimethylammonium methylsulfate dimethylcarbamate or Benzenamimium, 3[[[(dimethylamino) carbonyl]oxy]-N,N,N trimethyl-methylsulfate

Molecular Formula/ Molecular Weight      C<sub>13</sub>H<sub>22</sub>N<sub>2</sub>O<sub>6</sub>S / 334.39 g/mol

Structure



Pharmacologic Class      Cholinesterase inhibitor (FDA Established  
Pharmacological Class)

## 2.6 Proposed Clinical Population and Dosing Regimen

Neostigmine Methylsulfate Injection is indicated to reverse the neuromuscular blocking effects of non-depolarizing muscle relaxants. According to the Sponsor's proposed labeling, the recommended dose regimen is intravenous bolus injection with a dose range of (b) (4) -0.07 mg/kg or up to 5 mg, whichever is less for both children and adults, titrated on an individual basis by monitoring of neuromuscular activities.

## 2.7 Regulatory Background

A New Drug Application was submitted by Fresenius Kabi for Neostigmine Methylsulfate Injection on December 2011 but the application was issued a Complete Response due to deficiencies at a manufacturing facility (Grand Island, NY). In the Complete Response letter dated January 2013, seven additional studies were listed that would have to be completed as Postmarketing Requirements if the application were approved. These included genotoxicity studies, reproductive and developmental toxicity studies and an extractables/leachables assessment. In the current submission, the Sponsor has submitted all of the required nonclinical studies with the exception of the extractables/leachables assessment. According to the Sponsor, the extractable leachable assessment is almost completed and will be submitted in the near future.

## 3 Studies Submitted

### 3.1 Studies Reviewed

- SDN 17 (10/14/2013):
  - Neostigmine Methylsulfate, USP: In vitro Mammalian Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL)
  - Neostigmine Methylsulfate, USP: In Vivo Micronucleus Assay in Rats
- SDN 20 (3/12/2014):

- Neostigmine Methylsulfate: An Intravenous Study of Fertility and Early Embryonic Development to Implantation in Rats
- Neostigmine Methylsulfate: An Intravenous Study for Effects on Embryo-fetal Development in Rats with a Toxicokinetic Evaluation
- Neostigmine Methylsulfate: An Intravenous Study for Effects on Embryo-fetal Development in Rabbits with a Toxicokinetic Evaluation
- Neostigmine Methylsulfate: Toxic Effects on Pre- and Postnatal Development, including Maternal function in Rats Following Intravenous Administration
- SDN 21 (7/11/2014)
  - Labeling

### 3.2 Studies Not Reviewed

N/A

### 3.3 Previous Reviews Referenced

See original review dated 8/28/2012 by this reviewer.

## 7 Genetic Toxicology

### 7.2 *In Vitro* Assays in Mammalian Cells

**Study title: Neostigmine Methylsulfate, USP: In Vitro Mammalian Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes (HPBL)**

Study no.:	AD66MF.341ICH.BTL
Study report location:	e-submission, SDN-17
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	01/25/2013
GLP compliance:	Yes, a signed GLP compliance statement was included in the report.
QA statement:	Yes
Drug, lot #, and % purity:	Neostigmine methylsulfate, batch LTNM1A1009, 99.6% purity.

### Key Study Findings

Neostigmine methylsulfate was negative in the chromosomal aberration assay in human peripheral blood lymphocytes in the presence and absence of metabolic activation system.

## Methods

Cell line:	Human peripheral blood lymphocytes (HPBL)
Concentrations in definitive study:	150, 250, and 334 mcg/mL
Basis of concentration selection:	Dose limit of 10 mM (334 mcg/mL for Neostigmine Methylsulfate)
Negative control:	0.9% saline
Positive control:	-S9, Mitomycin, 0.3 mcg/mL for 20 hours of exposure and 0.6 mcg/mL for 4 hours of exposure; +S9, Cyclophosphamide, 5 mcg/mL
Formulation/Vehicle:	0.9% saline
Incubation & sampling time:	4 hour incubation + 16 hour recovery, ±S9; 20 hour incubation, -S9.

## Study Validity

This study is considered valid based on the following;

- Adequate doses were tested (dose limit of 10 mM)
- At least 200 metaphase cells (100 per duplicate culture) were examined and scored for chromatid-type and chromosome-type aberrations.
- Results of positive and negative controls were within expected ranges.

## Results

- The test article was soluble in water at all concentrations tested.
- Test compound at concentrations up to 334 mcg/mL (10 mM) generated no mitotic inhibition.
- Test article produced no increased chromosomal structural or numerical aberrations relative to vehicle control (see the table below)

TABLE 7  
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
0.9% Saline	-S9	4	13.6	200	200	0.000	±0.000	0.0	0.0
Neostigmine MethylSulfate USP									
125	-S9	4	13.2	200	200	0.000	±0.000	0.0	0.0
250	-S9	4	13.6	200	200	0.005	±0.071	0.0	0.5
334	-S9	4	13.4	200	200	0.000	±0.000	0.0	0.0
MMC 0.6	-S9	4	7.2	200	100	0.270	±0.489	0.0	25.0**
0.9% Saline	+S9	4	11.2	200	200	0.000	±0.000	0.0	0.0
Neostigmine MethylSulfate USP									
125	+S9	4	9.4	200	200	0.005	±0.071	0.0	0.5
250	+S9	4	9.7	200	200	0.005	±0.071	0.0	0.5
334	+S9	4	8.1	200	200	0.010	±0.100	0.0	1.0
CP 5	+S9	4	6.2	200	100	0.170	±0.403	0.0	16.0**
0.9% Saline	-S9	20	13.9	200	200	0.000	±0.000	0.0	0.0
Neostigmine MethylSulfate USP									
125	-S9	20	13.2	200	200	0.000	±0.000	0.0	0.0
250	-S9	20	14.0	200	200	0.010	±0.100	0.0	1.0
334	-S9	20	12.2	200	200	0.000	±0.000	0.0	0.0
MMC 0.3	-S9	20	7.1	200	100	0.410	±0.698	0.0	36.0**

**Treatment:** Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

**Aberrations per Cell:** Severely damaged cells were counted as 10 aberrations.

**Percent Aberrant Cells:** \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.01$ ; using the Fisher's Exact test.

In conclusion, Neostigmine Methylsulfate did not induce chromosomal aberrations in the absence and presence of metabolic activation system.

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

#### Study title: *In Vivo* Micronucleus Assay in Rats

Study no: AD66MF.125M012ICH.BTL  
Study report location: e-submission, SDN-17  
Conducting laboratory and location:  (b) (4)  
Date of study initiation: 1/25/2013  
GLP compliance: Yes, a signed GLP compliance statement was included in the study report  
QA statement: Yes  
Drug, lot #, and % purity: Neostigmine Methylsulfate, Lot No. LTNM1A1009; purity 99.6%

#### Key Study Findings

Neostigmine methylsulfate did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in rat bone marrow.

#### Methods

Doses in definitive study: 0, 50, 100 and 200 mcg/kg in male rats  
Frequency of dosing: Once a day, administered on two consecutive days  
Route of administration: Intravenous injection  
Dose volume: 5 mL/kg  
Formulation/Vehicle: 0.9% saline  
Species/Strain: Sprague Dawley rats  
Number/Sex/Group: 24-hour sacrifice, 5 males/dose; 48-hour sacrifice, 5 males for control and high dose of 200 mcg/kg  
Satellite groups: None  
Basis of dose selection: Maximal Tolerated Dose (MTD) (3/3 males and 2/3 females at 250 mcg/kg; 3/3 males and 3/3 females at 300 mcg/kg died in in dose ranging study)  
Negative control: 0.9% saline  
Positive control: Cyclophosphamide single oral dose at 40 mg/kg

#### Study Validity

This study is valid based on the following:

- Adequate doses were tested (up to MTD).

- 2000 polychromatic erythrocytes (PCEs)/animal were counted for micronucleated PCEs (mnPCEs); 1000 total erythrocytes [PCEs + normal chromatic erythrocytes (NCEs)] were scored per animal to determine the proportion of PCEs as an index of bone marrow toxicity.
- Positive control induced a statistically significant increase in the incidence of mnPCEs. The number of mnPCEs in the vehicle control groups did not exceed the historical control range.

## Results

- One rat died at the high dose of 200 mcg/kg. Piloerection or convulsions were noted in animals dosed at 100 and 200 mcg/kg. All animals at 50 mcg/kg, and the vehicle and positive control groups appeared normal during the study.
- Neostigmine treatment did not induce significant bone marrow toxicity based on lack of appreciable reductions in PCEs/EC ratio.
- No statistically significant increase in the incidence of mnPCEs in the test-article treated groups was observed relative to the negative control group. The table below presents the summary results.

Table 3  
Summary of Bone Marrow Micronucleus Analysis  
Following a Single Intravenous Administration of Neostigmine Methylsulfate, USP  
in Male Hsd:SD Rats

Treatment (5 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of mnPCE/1000 PCE (Mean +/- SD)	Number of mnPCE/PCE Scored
0.9% Saline	M	24	5	0.593 ± 0.02	---	1.5 ± 0.61	15 / 10000
Neostigmine Methylsulfate, USP 50 µg/kg	M	24	5	0.633 ± 0.03	7	1.3 ± 1.04	13 / 10000
100 µg/kg	M	24	5	0.606 ± 0.02	2	1.0 ± 0.61	10 / 10000
200 µg/kg	M	24	5	0.593 ± 0.03	0	1.3 ± 0.91	13 / 10000
Cyclophosphamide 40 mg/kg	M	24	5	0.617 ± 0.05	4	16.9 ± 1.39	*169 / 10000
0.9% Saline	M	48	5	0.566 ± 0.08	---	1.6 ± 0.74	16 / 10000
Neostigmine Methylsulfate, USP 200 µg/kg	M	48	5	0.606 ± 0.03	7	1.8 ± 0.57	18 / 10000

\*Statistical significant increase compared to vehicle control  $p \leq 0.05$  (Kastenbaum-Bowman Tables)  
PCE: Polychromatic Erythrocytes; mnPCEs: Micronucleated Polychromatic Erythrocytes

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

#### Study title: Neostigmine Methylsulfate: An Intravenous Study of Fertility and Early Embryonic Development to Implantation in Rats

Study no.:	(b) (4) Study No. 1999-006
Study report location:	e-submission, SDN-20
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/24/2013
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	Neostigmine Methylsulfate, Batch LTNM1A1009, 99.6%

#### Key Study Findings

Intravenous administration of Neostigmine to rats at 0, 10, 25, and 50 mcg/kg/day resulted in no remarkable findings in fertility and early embryonic development, although treatment-related clinical signs of tremor/twitching and salivation were observed. The high dose of 50 mcg/kg was defined as the NOAEL for fertility and reproductive performance for both males and females.

## Methods

Doses: 0, 10, 25, and 50 mcg/kg/day  
Frequency of dosing: Daily  
Dose volume: 1 mL/kg  
Route of administration: IV (injected over 1-2 minutes)  
Formulation/Vehicle: 0.9% Sodium Chloride Injection, USP  
Species/Strain: SD rats  
Number/Sex/Group: 25/sex/dose  
Satellite groups: None  
Study design: Dosing began 28 days prior to pairing for the males, and 14 days prior to pairing for the females. Dosing of the males continued through the mating and post-mating period to euthanasia, while dosing of the females continued through the mating period to Gestation Day (GD) 7. Necropsy was performed on GD 13 for females and at termination of the study for males.

The high dose of 50 mcg/kg was selected based on a dose-ranging study (Protocol 1999-004) where 25, 50, 75 and 100 mcg/kg/day was tested and prostration, splayed limbs, constricted pupils, jerky head movements, and head tilt were observed at 75 and/or 100 mcg/kg, suggesting exaggerated pharmacological effects at these doses. Therefore, the selection of high dose of 50 mcg/kg was considered acceptable.

Deviation from study protocol: There were no significant deviations from the protocol that affects interpretation of the results.

## Observations and Results

### Mortality

One male in each of 25 mcg/kg and 50 mcg/kg groups were found dead on Study Days 15 and 35, respectively. The causes of deaths were not identified. Histopathological findings were limited to red discoloration in multiple lobes of the lung and thyroid/parathyroid in the rat from the 25 mcg/kg group. In the absence of mortality in other males or females at 25 and 50 mcg/kg, these two deaths were not considered test article related.

### Clinical Signs

Tremor/twitching were observed in all animals at 25 and 50 mcg/kg within 15 minutes following dosing. Additionally, five males in each of 25 and 50 mcg/kg dosing groups were observed with salivation.

**Body Weight**

Not significantly affected

**Food Consumption**

No remarkable findings

**Toxicokinetics**

Not performed

**Dosing Solution Analysis**

**Concentrations of all samples of dose formulations were in the range of 102.3% to 105.5% of the nominal concentrations.**

**Necropsy****Fertility Parameters**

Reproductive and fertility indices are summarized in the table below. Estrous cycle, mating index (male and female), fertility index (male and female) were not affected by the test article.

There were no treatment-related findings in the numbers of corpora lutea, implantation sites, viable fetuses, resorptions, pre- and post-implantation loss.

	<b>0 mcg/kg/day</b>	<b>10 mcg/kg/day</b>	<b>25 mcg/kg/day</b>	<b>50 mcg/kg/day</b>
Mating Index	100%	100%	100%	100%
Fertility Index	96%	96%	100%	100%
Corpora lutea/litter	16.1	15.4	16.4	15.4
Implantation sites/litter	14.9	14.3	14.5	14.0
Preimplantation loss	6.5%	6.1%	9.9%	7.5%
Early + Late resorptions	1.1%	0.6%	0.6%	0.6%
Live fetuses/litter	13.9	13.8	14.0	13.4
Post-implantation loss	7.0%	3.9%	4.0%	4.6%

Sperm evaluation (sperm motility, concentration and morphology) revealed no treatment related findings.

<b>Parameter</b>	<b>0 mcg/kg/day</b>	<b>10 mcg/kg/day</b>	<b>25 mcg/kg/day</b>	<b>50 mcg/kg/day</b>
Sperm count per Cauda Epididymis $10^8$	2.98	2.96	2.90	3.03
Sperm motility (% motile)	85.1	88.8	83.1	86.6
Sperm morphology (% abnormal)	4.3	5.1	5.7	4.9

There were no remarkable findings in macroscopic examinations and organ weights.

## 9.2 Embryonic Fetal Development

### Study title: Neostigmine Methylsulfate: An Intravenous Study for Effects on Embryo-Fetal Development in Rats with a Toxicokinetic Evaluation

Study no.:	(b) (4) Study No. 1999-007
Study report location:	e-submission, SDN-20
Conducting laboratory and location:	(b) (4)
Date of study initiation:	01/24/2013
GLP compliance:	Yes, a signed GLP compliance statement was included in the study report
QA statement:	Yes
Drug, lot #, and % purity:	Neostigmine Methylsulfate, LTNM1A1009, purity 100% (HPLC).

### Key Study Findings

Intravenous administration of neostigmine methylsulfate to pregnant rats at doses of 0, 10, 25, and 50 mcg/kg/day from Gestation Days 6 to 17 resulted in no treatment-related findings in fetal body weights, external, visceral, or skeletal evaluations although tremors were observed in all animals at 25 and 50 mcg/kg following dosing. The NOAEL was 10 mcg/kg for maternal toxicity based on the tremor findings and 50 mcg/kg (high dose) for developmental/fetal toxicity.

### Methods

Doses:	0, 10, 25, and 50 mcg/kg/day
Frequency of dosing:	Daily
Dose volume:	1 mL/kg
Route of administration:	Intravenously (bolus injection over 10-15 seconds)
Formulation/Vehicle:	0.9% Saline
Species/Strain:	CD <sup>®</sup> [CrI:CD <sup>®</sup> (SD)] rats
Number/Sex/Group:	25 pregnant females/dose
Satellite groups:	TK animals 9 Pregnant females/dose (3 for control)
Study design:	Animals were treated daily from Gestation Day (GD) 6 to GD 17, and necropsied on GD 20. Standard observations and examinations were included in the main study animals and toxicokinetic data were collected from the satellite animals.
Deviation from study protocol:	No protocol deviations occurred affecting the quality or integrity of the study.

### Observations and Results

**Mortality**

None

**Clinical Signs**

Tremors were observed in all animals at 25 and 50 mcg/kg within 15 minutes following dosing. This finding is treatment related.

**Body Weight**

A slightly lower body weight gain from GD 6 to GD 9 (11.7 grams versus 16.0 grams in control) was noted in animals at 50 mcg/kg (mean 27% reduction). However, due to the transient in nature, lack of impact in mean gestation body weight and lack of associated findings in food consumption, it is not clear if this finding is treatment related.

**Food Consumption**

No remarkable findings

**Toxicokinetics**

Systemic exposure ( $C_{max}$ , which is  $C_0$  in the current case, and  $AUC_{0-1h}$ ) were increased with increasing dose. However, the manner of increased exposure was more than dose-proportional following the first day of dosing (GD 6), but dose-proportional or less than dose-proportional following the last day of dosing (GD 17). This inconsistent dose-relationship might reflect the variability, especially considering the short plasma  $T_{1/2}$  (0.136-0.264 hours) of neostigmine. The following table presents the details.

Dose, mcg/kg	Gestation Day	$C_0$ , ng/mL	$AUC_{0-1h}$ , hg.h/mL	$T_{1/2}$ , hour
10	6	7.84	0.992	0.160
	17	30.5	1.51	0.136
25	6	121	5.52	0.182
	17	24.7	2.72	0.164
50	6	371	14.6	0.207
	17	80.7	5.86	0.264

**Dosing Solution Analysis**

Dosing solution analysis revealed mean concentrations that ranged between 105% to 110% of the nominal concentrations and are deemed adequate.

## Necropsy

### Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were no treatment related findings in pregnancy index, gravid uterine weights at GD 20, and uterine and ovarian parameters (corpora lutea count, number of implantation sites, viable fetuses, litter size, pre- and post-implantation loss, and number of resorptions).

Mean values of maternal and development observations at uterine examination

<b>Dose, mcg/kg</b>	<b>0</b>	<b>10</b>	<b>25</b>	<b>50</b>
Pregnancy index, %	100	92	100	96
Gravid uterine weight, g	74.4	76.3	73.0	72.0
Corpora lutea count	14.6	13.6	13.2	13.5
Implantation sites	12.5	12.6	12.1	12.5
Pre-implantation loss, %	13.45	6.86	8.30	6.66
Post-implantation loss, %	3.53	3.69	5.81	9.24
Viable fetuses	12.0	12.1	11.4	11.5
Resorption (early+late)	0.5	0.5	0.8	1.0

Note, other than pregnancy index and gravid uterine weight, all other parameters were expressed as mean value per animal.

### Offspring (Malformations, Variations, etc.)

Fetal sex ratio and fetal body weight were not affected by neostigmine treatment.

There were no external or visceral malformations observed in fetuses from dams treated with 25 or 50 mcg/kg neostigmine. Two fetuses from two different dams at low dose were observed with external and visceral malformations (one fetus had microcephaly, open eye, malpositioned eyes, micrognathia, small tongue, facial cleft, absent papillae, malpositioned and small pinnae, small tongue, mischafen cerebral hemisphere, olfactory lobe and eye lens, cleft palate, malpositioned esophagus, absent innominate artery and retroesophageal subclavian artery; a second fetus had misshaped jaw, complete situs inversus of abdomen and thoracic cavity). Based on the lack of dose-relationship and sporadic incidence of these findings, these malformations and variations were not considered to be treatment related.

Skeletal examinations exhibited a malformation (bent scapula) in one single fetus at 50 mcg/kg. In the absence of other skeletal findings in this fetus, or similar findings in other fetuses, this isolated incidence of skeletal malformation was not considered to be treatment related.

All other findings were observed throughout the treated groups, at a low frequency, similar in incidence to controls or have been seen in recent historical control data for the conducting laboratory and were not considered test article related.

**Maternal Macroscopic Observations**

No remarkable findings

**Study title: Neostigmine Methylsulfate: An Intravenous Study for Effects on Embryo-Fetal Development in Rabbits with a Toxicokinetic Evaluation**

Study no.: (b) (4) Study No. 1999-008  
Study report location: e-submission, SDN-20  
Conducting laboratory and location: (b) (4)  
Date of study initiation: 01/24/2013  
GLP compliance: Yes, a signed GLP compliance statement was included in the study report  
QA statement: Yes  
Drug, lot #, and % purity: Neostigmine Methylsulfate, LTNM1A1009, purity 100% (HPLC).

**Key Study Findings**

Intravenous administration of neostigmine methylsulfate to pregnant rabbits at doses of 0, 10, 25, and 40 mcg/kg/day from Gestation Days 6 to 18 resulted in no treatment related findings in fetal body weights, external, visceral, or skeletal evaluations although tremors were observed in all animals at 25 and 40 mcg/kg following dosing. The NOAEL was 10 mcg/kg for maternal toxicity based on the tremor findings and 40 mcg/kg (high dose) for developmental/fetal toxicity. There was no evidence of teratogenic effects at dose levels up to 40 mcg/kg/day.

**Methods**

Doses: 0, 10, 25 and 40 mcg/kg/day  
Frequency of dosing: Daily  
Dose volume: 1 mL/kg  
Route of administration: Intravenously (bolus injection over 10-15 seconds)  
Formulation/Vehicle: 0.9% Saline  
Species/Strain: CD<sup>®</sup>[CrI:CD<sup>®</sup> (SD)] rats  
Number/Sex/Group: 23 pregnant females/dose  
Satellite groups: TK animals: 8 pregnant females/dose (3 for control)  
Study design: Animals were treated daily from Gestation Day (GD) 6 to GD 18, and necropsied on GD 29. Standard observations and examinations were included in the main study animals and toxicokinetic data were collected from the satellite animals.

Group	Dose Level* µg/kg/day	Total Number of Time-mated Females = 124		
		Number of animals assigned on GD 0	Main Study	Toxicokinetic
1	0	23	23	-
2	Low Dose	23	23	-
3	Mid Dose	23	23	-
4	High Dose	23	23	-
5	0	8	-	8
6	Low Dose	8	-	8
7	Mid Dose	8	-	8
8	High Dose	8	-	8

\* Actual dose levels will be added by amendment and determined from results from the (b) (4) Dose-Range Finding Study (1999-005).

The high dose of 40 mcg/kg was selected based on a dose-ranging study (Protocol 1999-005) where 10, 25, 50, 75 were tested and mortality (2/6 main study and 3/6 TK study animals) was observed at 75 mcg/kg with prior to death clinical signs of rapid breathing, convulsion, lacrimation, splayed limbs, constricted pupils, salivation and whole body twitching. The selection of 40 mcg/kg as the high dose in this study is acceptable.

Deviation from study protocol: No protocol deviations occurred that affected the quality or integrity of this study.

## Observations and Results

### Mortality

None

### Clinical Signs

The primary treatment related clinical signs were tremors that were observed in majority of animals at 25 and 40 mcg/kg within 15 minutes following dosing. A few other treatment related findings including decreased activity, prostration, splayed limbs, pupillary reflex diminished, breathing abnormalities and/or salivation were also observed in one or two animals at 40 mcg/kg.

### Body Weight

No remarkable findings

### Food Consumption

No remarkable findings

### Toxicokinetics

Measured on both first day and last day of treatment, systemic exposure ( $C_{max}$ , which is  $C_0$  in the current case, and  $AUC_{0-1h}$ ) were increased with increasing dose in a slightly more than dose proportional manner. The systemic exposures following the last day treatment (Gestation Day 18) were 14-80% higher than that following the first day treatment (Gestation Day 6). This increased exposure might represent a normal variability rather than dose accumulation based on the  $T_{1/2}$  of 0.17 hours. The table presents the details.

Dose, mcg/kg	Gestation Day	$C_0$ , ng/mL	$AUC_{0-1h}$ , hg.h/mL	$T_{1/2}$ , hour
10	6	12.6	1.98	NR
	18	23.9	2.84	0.175
25	6	39.7	6.30	0.177
	18	45.5	8.36	0.171
40	6	96.3	13.6	0.172
	18	176	16.2	NR

### Dosing Solution Analysis

Dosing solution analysis revealed mean concentrations range of 103.3% to 110% of the nominal concentrations.

### Necropsy

#### Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There were no treatment related findings in pregnancy index, gravid uterine weights at GD 29, and uterine and ovarian parameters (corpora lutea count, number of implantation sites, viable fetuses, litter size, pre- and post-implantation loss, and number of resorptions).

Mean values of maternal and development observations at uterine examination

Dose, mcg/kg	0	10	25	50
Pregnancy index, %	87	95.7	95.7	91.3
Gravid uterine weight, kg	0.539	0.491	0.505	0.490
Corpora lutea count	10.6	9.9	10.1	9.8
Implantation sites	9.7	8.7	9.4	8.9
Pre-implantation loss, %	7.63	11.98	7.27	10.10
Post-implantation loss, %	3.32	6.32	4.92	2.06
Viable fetuses	9.4	8.2	8.9	8.7
Resorption (early+late)	0.4	0.5	0.5	0.2

Note, other than pregnancy index and gravid uterine weight, all other parameters were expressed as mean value per animal.

### Offspring (Malformations, Variations, etc.)

Fetal sex ratios (% males) in treated groups (46.1% - 55.0%) were comparable to controls (50%).

Fetal body weights in treated groups (average 41.31-43.64 g) were comparable to controls (41.65 g).

There were no treatment related findings in external, visceral, or skeletal examinations. A few incidences of malformations in external, visceral, or skeletal examinations were observed in treatment groups (see the table below). However, all these findings were within recent historical control range of the study laboratory.

<b>Observation</b>		0	10 mcg/kg	25 mcg/kg	50 mcg/kg
	No. litters evaluated	20	22	22	21
	No. fetuses evaluated	187	180	195	182
<b>External findings</b>	<b>Total Malformations</b>				
	No. Litters (%)	1 (5.0)	0 (0.0)	2 (9.1)	1 (4.8)
	No. Fetuses (%)	1 (0.5)	0 (0.0)	1 (1.0)	1 (0.5)
	<b>Total Variations</b>				
	No. Litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	No. Fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<b>Visceral findings</b>	<b>Total Malformations</b>				
	No. Litters (%)	3 (15.0)	2 (9.1)	0 (0.0)	1 (4.8)
	No. Fetuses (%)	3 (1.6)	2 (1.1)	0 (0.0)	1 (0.5)
	<b>Total Variations</b>				
	No. Litters (%)	7 (35.0)	6 (27.3)	6 (27.3)	8 (38.1)
	No. Fetuses (%)	8 (4.3)	9 (5.0)	8 (4.1)	11 (6.0)
<b>Skeletal findings</b>	<b>Total Malformations</b>				
	No. Litters (%)	6 (30.0)	9 (40.9)	4 (18.2)	7 (33.3)
	No. Fetuses (%)	12 (6.4)	11 (6.1)	6 (3.1)	9 (4.9)
	<b>Total Variations</b>				
	No. Litters (%)	20 (100.0)	22 (100.0)	21 (95.5)	20 (95.2)
	No. Fetuses (%)	100 (53.5)	92 (51.1)	90 (46.2)	87 (47.8)

### Maternal Macroscopic Observations

No remarkable findings

### 9.3 Prenatal and Postnatal Development

#### **Study title: Neostigmine Methylsulfate: Toxic Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Following Intravenous Administration**

Study no.: (b) (4) Study No. 1999-012  
Study report location: e-submission, SDN-20  
Conducting laboratory and location: (b) (4)  
Date of study initiation: 01/24/2013  
GLP compliance: Yes, a signed GLP compliance statement was included in the study report  
QA statement: Yes  
Drug, lot #, and % purity: Neostigmine Methylsulfate, LTNM1A1009, purity 100% (HPLC).

#### **Key Study Findings**

Intravenous administration of neostigmine methylsulfate (0, 10, 25, and 50 mcg/kg/day) to pregnant rats from Gestation Day 6 to Gestation Day 20 resulted in clinical signs of tremors observed within 15 minutes following dosing, and red material around the eyes, nose and/or mouth in animals at 25 and 50 mcg/kg/day. However, no other treatment related effects were observed in the parental females (survival, body weight and food consumption), parturition, F<sub>1</sub> litter size or in the evaluation of F<sub>1</sub> pups preweaning (sex ratios, survival to weaning, body weights, clinical signs, behavior, developmental and sensory evaluations) or in F<sub>1</sub> pups postweaning (survival, growth, sexual maturation, motor activity, learning and memory, reproductive performance and fertility). Likewise, no treatment related effects was observed in macroscopic evaluations of the F<sub>0</sub> and F<sub>1</sub> animals. The NOAEL was 10 mcg/kg for maternal toxicity and 50 mcg/kg for reproductive performance of the parental females and F<sub>1</sub> offspring development.

## Methods

Doses: 0, 10, 25, and 50 mcg/kg  
Frequency of dosing: Daily  
Dose volume: 1 mL/kg  
Route of administration: IV  
Formulation/Vehicle: 0.9% Sodium Chloride  
Species/Strain: CD<sup>®</sup>[CrI:CD<sup>®</sup> (SD)] rats  
Number/Sex/Group: 25/sex/group  
Satellite groups: None  
Study design: The test article was administered daily to pregnant female rats via a slow bolus intravenous injection over 10-15 seconds. Dosing began on Gestation Day 6 and continued through to include Lactation Day 20. The F<sub>1</sub> offspring were potentially exposed to the test article in utero and as neonates during the lactation period but were not dosed directly. Litters were housed with the dams for 3 weeks after birth (Lactation Days 0-21, or LD 0-21). The dams and litters were observed daily for survival and behavioral alterations. Pups were individually weighed and examined externally on LD 0, 4, 7, 14, and 21. On LD 21, F<sub>0</sub> females were necropsied and the number of uterine implantation scars was recorded. The F<sub>1</sub> generation was examined for behavioral and developmental indices (static righting reflex and pinna detachment at LD 2; cliff aversion at LD 11, eye opening at LD 13, air drop righting reflex at LD 16, behavior and neurology at LD 21; auditory response at Postnatal Day 22; vaginal opening at Postnatal Day 28 and after; preputial separation and motor activity at Day 35 of age, step-through passive avoidance test initiated between 70 and 85 days of age). F<sub>1</sub> reproductive/fertility assessment was conducted by pairing F<sub>1</sub> pups at age of 80 days or older for 20 days. On Gestation Day 13, each F<sub>1</sub> female was euthanized and the numbers of embryos, resorptions, and implantations, as well as the number of corpora lutea were recorded. F<sub>1</sub> males were necropsied after completion of the Cesarean section examination of F<sub>1</sub> females.

		Total Number of P Females = 100, F <sub>1</sub> Females = 100, and F <sub>1</sub> Males = 100					
Group	Dose Level (µg/kg/day)	Mated P Females Assigned to Study on GD 0 <sup>a</sup>	Number of Selected F <sub>1</sub> Pups Assigned to Study		P Females Necropsy	F <sub>1</sub> Animals Necropsy	
			F	M		F	M
1	0	25	25	25	25	25	25
2	Low Dose 10	25	25	25	25	25	25
3	Mid Dose 25	25	25	25	25	25	25
4	High Dose 50	25	25	25	25	25	25

<sup>a</sup>P females are time mated at receipt

Deviation from study protocol: No deviations affecting the quality or integrity of the study occurred.

## Observations and Results (Optional Table)

## REPRODUCTION GLOSSARY

**Weaning** - The day on which an animal is separated from its mother.

**Fecundity Index or Pregnancy Index - Males**

$$\frac{\text{No. males impregnating a female}}{\text{No. males with evidence of mating}} \times 100$$

**Fecundity Index or Pregnancy Index - Females**

$$\frac{\text{No. females pregnant}}{\text{No. females with evidence of mating}} \times 100$$

**Females Delivering Litters (%)**

$$\frac{\text{No. females delivering litters}}{\text{No. females pregnant}} \times 100$$

**Females with All Stillborn (%)**

$$\frac{\text{No. litters with all stillborn pups}}{\text{No. females delivering litters}} \times 100$$

**Females with Stillborn Pups (%)**

$$\frac{\text{No. litters with at least 1 stillborn pup}}{\text{No. females delivering litters}} \times 100$$

**Fertility Index - Males**

$$\frac{\text{No. males impregnating a female}}{\text{No. males paired}} \times 100$$

**Fertility Index - Females**

$$\frac{\text{No. females pregnant}}{\text{No. females paired}} \times 100$$

**Gestation Index -**

$$\frac{\text{No. females delivering at least 1 live pup}}{\text{No. females pregnant}} \times 100$$

**Lactation Index - Day 21**

$$\frac{\text{No. pups surviving 21 days (weaning)}}{\text{No. live pups at 4 days (postculling)}} \times 100$$

**Live Birth Index -**

$$\frac{\text{No. live pups at birth}}{\text{No. pups born}} \times 100$$

**Mating Index - Males**

$$\frac{\text{No. males with evidence of mating}}{\text{No. males paired}} \times 100$$

**Mating Index - Females**

$$\frac{\text{No. females with evidence of mating}}{\text{No. females paired}} \times 100$$

**Stillborn Index -**

$$\frac{\text{No. stillborn pups}}{\text{No. pups born}} \times 100$$

**Viability Index - Day 4**

$$\frac{\text{No. pups surviving 4 days (preculling)}}{\text{No. live pups at birth}} \times 100$$

F<sub>0</sub> Dams

- Survival: All F<sub>0</sub> dams survived to scheduled termination.
- Clinical signs: All females at 25 and 50 mcg/kg exhibited tremors within 15 minutes following dosing. Additionally, higher incidence of red material around the eyes, nose and/or mouth was noted in the 50 mcg/kg group.
- Body weight: Mean gestation and lactation body weights in the treated groups were comparable to that of control.
- Food consumption: Not affected
- Uterine content: No treatment related findings (see below)

<b>Dose mcg/kg</b>	<b>0</b>	<b>10</b>	<b>25</b>	<b>50</b>
Pregnancy rate, %	100	100	100	96
Gestation length, days	21.6	21.7	21.7	21.8
Litter size at Day 0	12.0	12.0	11.9	11.9
Liveborn	12.0	12.0	11.8	11.8
Total implantation scars	12.4	12.5	12.3	12.5

- Necropsy observation: No remarkable findings
- Toxicokinetics: Not performed
- Dosing Solution Analysis: All formulation concentrations were in a range of 103.7% to 107.6% of nominal concentrations, based on the dosing solution analysis.

F<sub>1</sub> Generation

- Survival: Not affected (viability index-percentage see the table below)
- | <b>Dose mcg/kg</b> | <b>0</b> | <b>10</b> | <b>25</b> | <b>50</b> |
|--------------------|----------|-----------|-----------|-----------|
| Sex ratio, % males | 50.12    | 49.00     | 47.41     | 50.24     |
| Viability index    | 99.42    | 97.43     | 99.17     | 97.82     |
| Lactation index    | 100      | 99.50     | 99.50     | 100.00    |
- Clinical signs: No remarkable findings were observed during the 21-day lactation period and 1-week post weaning period.
- Body weight: No treatment related effects were observed during lactation, post weaning Day 28, pre mating, pairing (started at least 80 days of age) and post mating, as well as during gestation (females).
- Food consumption: No anomalies reported
- Physical development: No remarkable findings in F<sub>1</sub> behavioral, sensory, or developmental evaluation including sexual maturation assessment.
- Neurological assessment: No treatment-related findings in motor activity (basic movements, fine movements, rearing and total

- distance), and in learning and memory evaluations (passive avoidance test).
- Reproduction: No treatment-related findings were observed on mating performance and fertility of the F<sub>1</sub> animals. Mating, fertility and fecundity indices in the treated groups (males and females) were comparable to controls and within the range of recent historical control data for the laboratory. F<sub>1</sub> uterine examination showed no treatment-related effects (number of corpora lutea, implantation sites, viable fetuses, resorptions and pre- and post-implantation loss)
- Other: F<sub>1</sub> macroscopic examinations showed no remarkable findings.

## 11 Integrated Summary and Safety Evaluation

In response to the Division's complete response letter for the Sponsor's original NDA submission, the Sponsor has submitted a complete response package including updated labeling where nonclinical findings from their newly completed genotoxicity and reproductive and developmental toxicology studies were reflected.

### Genotoxicity:

The two genotoxicity studies, an in vitro chromo aberration assay in human peripheral blood lymphocytes and an in vivo rat bone marrow micronucleus assay were reviewed and were concluded negative findings in both studies.

Based on these negative findings and negative findings in Ames test that was previously reviewed (9/18/2013), neostigmine methylsulfate is not mutagenic or clastogenic.

### Reproductive and Developmental Toxicology:

A standard battery of reproductive and developmental toxicology studies were submitted. All studies used intravenous route to administer neostigmine methylsulfate as a daily bolus dose. The dose levels were 0, 10, 25, and 50 mcg/kg in rats; 0, 10, 25, and 40 mcg/kg in rabbits. The high dose tested, 50 mcg/kg in rats, 40 mcg/kg in rabbits were considered acceptable based the treatment-related clinical findings of tremor/twitch following dose administration. There were no treatment-related adverse findings in the fertility and early embryonic development study in rats, embryonic fetal development study in rats and rabbits, or the pre- and post-natal development study in rats. NOAELs for reproductive toxicity were defined as 50 mcg/kg for the rat studies and 40 mcg/kg for the rabbit study. These NOAELs represent human equivalent doses (on a mg/m<sup>2</sup> basis) of 8.06 mc/kg (rat data) and 12.9 mcg/kg (rabbit data). Compared to the maximum recommended human dose of 5 mg (83 mcg/kg for a 60 kg human body), these NOAELs are 6.5-10.3 times lower. Therefore, these negative findings in reproductive toxicology studies are of limited clinical relevance.

According to the above findings in genotoxicity studies and reproductive and embryo-fetal developmental studies, neostigmine methylsulfate injection product labeling is recommended to include the language as presented earlier (see 1.1.3 Labeling).

Regarding the leachable/extractable assessment for the container stopper that is exposed to (b) (4) phenol, the Sponsor has not submitted the study report and as a result the Applicant will be issued a postmarketing requirement to address this issue.

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/s/  
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HUIQING HAO  
12/17/2014

RICHARD D MELLON  
12/17/2014

I concur with Dr. Hao's recommendation that from a nonclinical pharmacology toxicology perspective, NDA 203629 may be approved with the recommended PMR.

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

**SECONDARY PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: 203629  
Supporting document/s: SDN 0, 4, 10  
Applicant's letter date: 12/28/2011, 3/21/2012, 8/29/2012  
CDER stamp date: 12/29/2011, 3/21/2012, 8/29/2012  
Product: Neostigmine Methylsulfate Injection  
Proposed Indication: Reversal of nondepolarizing neuromuscular blocking agents, [REDACTED] (b) (4)  
[REDACTED]  
[REDACTED]  
Applicant: APP Pharmaceuticals LLC  
Review Division: Anesthesia, Analgesia, and Addiction Products  
Reviewer: R. Daniel Mellon, Ph.D.  
Supervisor/Team Leader: Bob A. Rappaport, M.D.  
Division Director: Bob A. 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 203629 are owned by APP Pharmaceuticals LLC or are data for which APP Pharmaceuticals LLC has obtained a written right of reference. Any information or data necessary for approval of NDA 203629 that APP Pharmaceuticals LLC 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 203629.

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## 1 Executive Summary

The primary pharmacology toxicology review was completed by Dr. Huiqing Hao. Dr. Hao originally recommended a complete response based on the lack of adequate genetic toxicology data for the parent and drug substance impurities. A subsequent submission from the Sponsor (8/29/2012) addressed this concern. Based on the second review from Dr. Hao, she is recommending that the application can be approved at this time pending agreement on labeling and with post-marketing requirements (PMRs). I concur with this approval recommendation and with the recommendation for the PMRs.

As discussed in the 2009 preIND meeting with the Sponsor, 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. As noted in her reviews, adequate data were available to support the safety of the container closure system, the drug substance impurity specifications, and the drug product degradant specifications. In terms of excipient safety qualification, the total daily dose of the preservative phenol via this drug product formulation does exceed that of previously approved drug products that are administered as a single bolus injection; however, we recognize that previous clinical experience exists that may justify the safety in this product (see medical officer review).

As noted in the preIND meeting minutes from 2009, 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 unless adequate data could be identified in the literature to inform labeling. Based on the lack of adequate data in the published literature to inform labeling, these studies are recommended as post-marketing requirements.

### 1.1 Introduction

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 intestinal 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 appendix for summary table of NDAs containing neostigmine). Neostigmine is an acetylcholinesterase inhibitor that has also 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 compete with and displace neuromuscular blocking agents. APP's neostigmine methylsulfate injection is currently a marketed-unapproved drug. The Agency met with APP on December 22, 2009 to discuss the requirements to support an NDA submission for their drug. At that time, the Division informed APP that due to the long history of human use of the drug, nonclinical studies may not be necessary to support the safety of their neostigmine drug substance for their NDA. However, we clearly noted that nonclinical studies may be necessary to support the safety of any novel excipients, leachables, impurities in the drug substance, or

degradants in the drug product if the levels exceeded established guidelines. We also noted that if the impurities or degradants contain structural alerts for mutagenicity, they would be required to be reduced to not more than 1.5 mcg/day as outlined in the Draft FDA Guidance to Industry titled “Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches.” At that time, we also noted that there did not appear to be any genetic toxicology data or reproductive and developmental toxicology data for neostigmine in the published literature to inform product labeling. Although normally required for approval, given the extensive clinical use of the drug, we noted that the studies would likely be post-marketing requirements unless APP could identify adequate data for labeling.

The NDA was received on December 29, 2011. No new nonclinical studies were submitted at that time. Two study reports, an in vitro mutagenicity study for neostigmine (b) (4) were submitted on August 29, 2012.

## 1.2 Brief Discussion of Nonclinical Findings

With the exception of a single genetic toxicology study report for neostigmine, no new toxicology data were submitted for this drug substance. Although the Sponsor conducted a literature search, the data in the published domain primarily includes studies describing the pharmacodynamic effects of the drug. There were no published studies that could be described as adequate acute or repeat-dose toxicology studies, genetic toxicology studies, or reproductive and developmental toxicology studies that could be used to provide nonclinical safety support or inform the labeling.

There are three main nonclinical review issues identified by Dr. Hao in her review of this submission: (1) drug substance impurities with structural alerts for mutagenicity, (2) safety justification for the levels of the excipient phenol in the drug product, and (3) potential leachables/extractables from the container closure system due to the presence of phenol in this drug product formulation. Each of these will be summarized below and my recommendations regarding their impact on the application.

### Drug Substance Impurities

The Sponsor did not specifically discuss the potential for structural alerts in the drug substance impurities or the parent; rather they proposed to follow the current USP specifications. The structures and proposed specifications for the drug substance impurities are summarized in the table below. Upon review of the structures, we noted that all of the potential impurities contain structural alerts for mutagenicity. In fact, neostigmine itself contains structural alerts for mutagenicity as shown by the circled chemical moieties in the table below.

**Table 1: Drug Substance Impurities**

Impurity	Structure	Proposed specification	Reviewer Comment
----------	-----------	------------------------	------------------

Impurity	Structure	Proposed specification	Reviewer Comment
Neostigmine methylsulfate	(b) (4)	Active Drug Substance	The active drug substance contains two structural alerts for mutagenicity that are circled in red.



Impurity	Structure	Proposed specification	Reviewer Comment
(b) (4)			
Any Other Individual Impurity		NMT (b) (4) %	
Total Impurity		NMT (b) (4) %	

We requested a computational toxicology Qualitative Structure Activity Relationship (QSAR) evaluation, which is reproduced in the Appendix of Dr. Hao's first review. As she notes, all of the impurities were predicted to be positive in the Ames assay and one of the compounds actually has been reported to be positive in this assay. The lack of data for neostigmine and the structural alerts identified was discussed with the Sponsor in a teleconference on April 13, 2012. As there were no genetic toxicology data for neostigmine alone, and similar if not identical chemical moieties in the impurities are also in the parent, we recommended that they either tighten the specifications to result in exposure of NMT 1.5 mcg/day or conduct an Ames assay to determine if the impurities were genotoxic or not. At the current specifications, a person would be exposed to (b) (4) mcg/day of potentially genotoxic impurities that presumably do not contribute to the efficacy of the drug product and therefore only contribute risk. It is likely that these impurities are present in the currently marketed unapproved drug product supplied by APP. After evaluation of the information, the Sponsor indicated that they would pursue qualification via genetic toxicology data and submit the studies in August of 2012. On August 29, 2012, Ames assays for both neostigmine drug substance and drug product were

submitted. Upon review of these studies, Dr. Hao concluded that neostigmine did not demonstrate evidence of mutagenic potential.

As noted in the table above, the impurities either contain the exact same structural alert moiety or a very similar moiety that triggered the concern regarding genotoxicity as neostigmine itself. Therefore, the negative results in the Ames assays for neostigmine provide adequate safety justification for these impurities.

### Phenol as novel excipient

The Sponsor maintains that there are no novel excipients. As noted in Dr. Hao's review, 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 that perspective, 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 drug product to date. There are no intravenous toxicology studies for either phenol or this neostigmine formulation; therefore, there are technically inadequate data to justify the safety of the proposed bolus dose of phenol. However, the Division recognizes that this formulation has been marketed by APP 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 this NDA.

### Container Closure

In the preIND meeting held in 2009 the Division specifically noted that APP should provide adequate justification for the safety of the container closure system of this drug product. APP did not include a new leachable/extractable assessment for the container closure in the original NDA submission; rather they noted that a (b) (4) gray (b) (4) rubber serum stopper provided by (b) (4) has been used in many other aqueous drug products. Upon initial review, we were concerned that the phenol will alter the leachable profile and therefore, requested that an extractable/leachable study be completed and a toxicological risk assessment for the identified leachables be provided to support the safety of the container closure. This was communicated in the 74-day letter and the Sponsor originally indicated that the study results would be provided by July 31, 2012. (b) (4)

(b) (4) During the course of the review, Dr. Hao was able to identify two other FDA-approved intravenous generic drug products that also contain (b) (4) % phenol and use the same (b) (4) stopper in their container closure system. Therefore, since the Agency appears to have approved other intravenous phenol-containing drug products that employ the same container closure system, we cannot consider this an approval issue for this NDA at this time. A

(b) (4)

As we have been able to identify at least two ANDAs for intravenous drug products that were approved by the Office of Generic Drugs that also contain phenol, the lack of an adequate extractable/leachable risk assessment cannot be deemed an approval issue. Nonetheless, the concern that the phenol may result in increased leachables over time remains and there do not appear to be adequate data by current standards to address this issue. Therefore, I concur with Dr. Hao that the Sponsor should provide an adequate extractable leachable study as a PMR.

### 1.3 Recommendations

#### 1.3.1 Approvability

**Approval.** From a nonclinical pharmacology toxicology perspective, adequate information has been provided to support approval of this NDA. If approved in this or a second cycle, post-marketing requirements for the remaining two genetic toxicology studies, the complete battery of reproductive and developmental toxicology studies, and an adequate extractable/leachable assessment of the container closure system are also recommended.

#### 1.3.2 Additional Non Clinical Recommendations

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 number 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.
7. Conduct an adequate extractable/leachable safety assessment for the (b) (4) gray (b) (4) rubber stopper used in your container closure system. This assessment must include controlled extraction studies to qualitatively and quantitatively determine the chemical species which may migrate into the dosage form using appropriate solvents that adequately represent the chemical characteristics of the drug product formulation, and leachable data from long-term stability studies (taking into consideration the proposed shelf-life) to determine if the identified/specified extractables also leach into the drug product over time, and a toxicological risk assessment justifying the safety of the extractables and leachables taking into consideration the maximum daily dose of the identified materials for this drug product. For your toxicological risk assessment, any leachable that contains a structural alert for mutagenicity should not exceed 1.5 mcg/day total daily exposure or be adequately qualified for safety. A toxicological risk assessment should be provided for any non-genotoxic leachable that exceeds 5 mcg/day.

### 1.3.3 Labeling

**Table 2: Labeling Recommendations**

Sponsor’s Proposed	Recommended Labeling	Rationale/Comment
(b) (4)	<b>Highlights</b> <b>Indication and Usage</b> Neostigmine Methylsulfate Injection USP, a <b>cholinesterase inhibitor</b> , is indicated for reversal of nondepolarizing neuromuscular blocking agents ...	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.
	<b>Use in Special Populations</b> <b>Pregnancy: No human or animal</b>	The Sponsor proposed nothing in the highlights, as there

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
	<p>data. Use only if clearly needed.</p>	<p>currently no data, (b) (4)</p>
<p>(b) (4)</p>	<p><b>8 USE IN SPECIFIC POPULATIONS</b></p> <p><b>8.1 Pregnancy</b> <i>Teratogenic effects.</i> <b>Pregnancy Category C</b></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>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 Rule.</p> <p>Language modified to accurately reflect the CFR requirements.</p>
<p>(b) (4)</p>	<p><b>12 CLINICAL PHARMACOLOGY</b></p> <p><b>12.1 Mechanism of Action</b></p> <p>Neostigmine is (b) (4) competitive cholinesterase inhibitor (b) (4)</p> <p>By reducing the breakdown of acetylcholine, neostigmine-induced increase in acetylcholine completes for the same binding sites as nondepolarizing neuromuscular junction blockers. (b) (4)</p>	<p>Pharmacodynamic and mechanism of action will be discussed further with the clinical pharmacology review team.</p>

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
<p>(b) (4)</p>	<p>(b) (4)</p> <p><b>12.2 Pharmacodynamics</b> (b) (4)</p> <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>(b) (4)</p> <p><b>13 NONCLINICAL TOXICOLOGY</b></p>	
	<p><b>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</b></p> <p>Mutagenesis. Neostigmine was not mutagenic in an in vitro bacterial reverse mutation assay (Ames test).</p> <p>Carcinogenesis. Long-term animal studies have not been performed to evaluate the carcinogenic potential, (b) (4) of (b) neostigmine (b) (4)</p> <p>Fertility. The potential effects of neostigmine on fertility have not been evaluated.</p>	

## 12 Appendix/Attachments

**Table 3: 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 and to document why neostigmine is not considered a new chemical entity.

### Reference List

1. 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.

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/s/  
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RICHARD D MELLON  
09/18/2012

**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  
Addendum**

Application number: 203629  
Supporting document/s: SDN-10  
Applicant's letter date: 08/29/2012  
CDER stamp date: 08/29/2012  
Product: Neostigmine Methylsulfate Injection  
Indication: Reversal agent to the neuromuscular blocking effects of non-depolarizing muscle relaxants  
Applicant: APP Pharmaceuticals LLC.  
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 A. Rappaport, M.D.  
Project Manager: Allison Meyer

*Template Version: September 1, 2010*

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# 1 Executive Summary

## 1.1 Introduction

This is an addendum to the first NDA review submitted on August 28, 2012. At that time, I recommended that the NDA not be approved due to the presence of structural alerts for mutagenicity in both the parent and drug substance impurities and the lack of adequate mutagenicity data for neostigmine and related impurities (original review, 8/28/2012). In the current submission, the Sponsor submitted bacterial mutation assay results for neostigmine and leachable data. This addendum reviews this new submission and provides an updated nonclinical recommendation.

## 1.2 Brief Discussion of Nonclinical Findings

An Ames test with neostigmine exhibited negative results in the presence and absence of metabolic activation system. Based on these data, neostigmine and associated impurities (with similar structural alert moiety) are concluded to be negative of mutagenic potential. Therefore, the proposed drug substance impurity specifications are acceptable.

(b) (4)



## 1.3 Recommendations

### 1.3.1 Approvability

As neostigmine is negative in the bacterial mutation assay, and no additional nonclinical approvability issues, this NDA is recommended to be approved.

### 1.3.2 Additional Non-Clinical Recommendations

The following are recommended as post marketing requirements:

- Conduct an in vitro assay using mammalian cells for chromosomal damage for neostigmine methylsulfate.
- Conduct an in vivo assay for chromosomal damage for neostigmine methylsulfate.

- Conduct a fertility and early embryonic development toxicology study in the rat model for neostigmine methylsulfate.
- Conduct an embryo-fetal developmental toxicology study using the rat model for neostigmine methylsulfate.
- Conduct an embryo-fetal developmental toxicology study using the rabbit model for neostigmine methylsulfate.
- Conduct a peri- and post-natal developmental toxicology study in the rat model for neostigmine methylsulfate.
- Conduct a well-controlled extractable study for the container closure system and based on the extractable profile obtain leachable profile for the product at the end of shelf life. Evaluate and justify the safety of the leachables that are associated with human exposure more than 1.5 mcg/day for genotoxic chemicals and more than 5 mcg/day for nongenotoxic chemicals.

### 1.3.3 Labeling

See the original review. The labeling should be updated to include the negative findings in the Ames assay.

## 2 Drug Information

### 2.1 Drug

CAS Registry Number 51-60-5

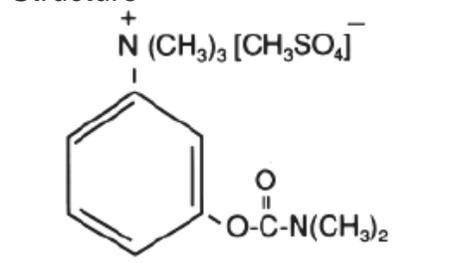
Generic Name Neostigmine methylsulfate

Code Name NA

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

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

## Structure



Pharmacologic Class

Cholinesterase inhibitor

## 2.6 Proposed Clinical Population and Dosing Regimen

Neostigmine Methylsulfate Injection is indicated to reverse the neuromuscular blocking effects of non-depolarizing muscle relaxants. According to the Sponsor's proposed labeling, the recommended dose regimen is intravenous bolus injection at dose range of (b) (4) -0.07 mg/kg for both children and adults, titrated on an individual basis by monitoring of neuromuscular activities.

## 2.7 Regulatory Background

See the original review dated 8/28/2012 by this reviewer.

## 3 Studies Submitted

### 3.1 Studies Reviewed

- Bacterial Mutation Assay of 5 mg/mL Neostigmine Methylsulfate Injection and 5 mg/mL Neostigmine Methylsulfate in Acetate Buffer
- (b) (4)

### 3.2 Studies Not Reviewed

NA

### 3.3 Previous Reviews Referenced

Original review, completed on 8/28/2012

## 7 Genetic Toxicology

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

**Study title:** Bacterial Mutation Assay of 5 mg/mL Neostigmine Methylsulfate Injection and 5 mg/mL Neostigmine Methylsulfate in Acetate Buffer

Study no.:	AD51SF-SG.502ICH.BTL
Study report location:	e-submission
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	05/24/2012
GLP compliance:	Yes, a signed GLP compliance statement was included in the report
QA statement:	Yes
Drug, lot #, and % purity:	5 mg/mL neostigmine methylsulfate injection, Lot R341-003Geno and 29993-13B, and 5 mg/mL neostigmine methylsulfate in acetate buffer, Lot 29993-5 and 299993-13A. No purity information obtained.

#### Key Study Findings

Neostigmine methylsulfate injection and neostigmine in acetate buffer without other excipients exhibited negative results in the Ames test with and without metabolic activation system.

## Methods

- Strains: *Salmonella typhimurium* tester strains TA98, TA100, TA1525 and TA1537 and *Escherichia coli* tester strain WP2uvr
- Concentrations in definitive study: 50, 150, 500, 1000, 2000 and 5000 mcg per plate were used in both studies with neostigmine methylsulfate injection and neostigmine methylsulfate in acetate buffer
- Basis of concentration selection: Dose limit of 5000 mcg/plate
- Negative control: Sodium acetate buffer, pH 5.9
- Positive control:

Strain	S9 activation	Positive control	Concentration, mcg/plate
TA98, TA1535, TA1537	+	2-aminoanthracene	1.0
TA100			2.0
WP2 uvrA			15
TA98	-	2-nitrofluorene	1.0
TA100, TA1535		Sodium azide	1.0
TA1537		9-aminoacridine	75
WP2uvrA		Methyl methanesulfonate	1000

Formulation/Vehicle: Neostigmine injection contains complete formulation of the drug (water, phenol 4.5 mg/mL, and sodium acetate 0.25 mg/mL), neostigmine acetate buffer contained neostigmine in acetate buffer without other excipients. Acetate buffer was used as vehicle control in both studies for neostigmine injection and neostigmine in acetate buffer.

Incubation & sampling time: Plate incorporation method was used. Incubation (exposure) time was 24-72 hours.

## Study Validity

This study was deemed valid based on the following:

- Adequate doses were used (up to 5000 mcg/plate)
- Triplicate cultures were used
- Revertant colonies were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity.
- Positive criteria: dose-related increase of revertants in at least two concentrations of test article; or peak response (revertant count) for tester strains TA1535 and TA1537 to be at least 3-times of control value; peak response for tester strain TA98, TA100 and WP2 *uvrA* to be at least 2.0-times of control values.

- Results of positive and negative control are in expected range.

### Study Outcome

- 5 mg/mL Neostigmine Methylsulfate Injection
  - No precipitation or background lawn toxicity was observed
  - Dosing formulation analysis indicated that the actual dose levels were 33.3-105.7% of their respective targeted concentration. A sample of 0.15 mg/mL concentration was confirmed to be 33.3% of target. Without specifying the affected dose levels (mcg/plate), the Sponsor stated that although the actual concentration of the low dose level was lower than expected, the critical top dose level was within 85.0 to 115% of target. This confirms the target theoretical drug concentration of 5 mg/mL and indicates that the regulatory-required top dose level was achieved (report p 18).
  - No positive mutagenic responses were observed in any tester strain with or without S9.
- 5 mg/mL Neostigmine Methylsulfate in acetate buffer only
  - No test compound precipitation or toxicity was observed.
  - Increased (2.8-4.0 fold) revertants in tester strain TA1537 in the presence and absence of S9 were observed. However, the increase was not dose related and the control values were on the low end of the acceptable range and the plate counts for all treated dose levels were within the historical range. Therefore, the increase of revertants was not considered to be indicative of mutagenic activity.
  - Dosing formulation analysis confirmed that actual doses were in a range of 97.5-107.9% of their respective targets.
  - No positive mutagenic responses were observed in any other strains with or without metabolic activation by S9.
  - Pre- and post-study analyses showed no significant changes in drug concentration or impurity profile. Therefore, the test article was stable in acetate buffer at a concentration of 5 mg/mL for the period of use in this study.

Based on the above study results, this reviewer agreed with the Sponsor, neostigmine is negative in the bacterial mutation assay.

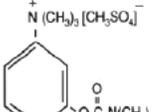
## 11 Integrated Summary and Safety Evaluation

### Mutagenicity of neostigmine and related impurities:

The current study report demonstrated negative mutagenic potential of neostigmine.

As discussed in the original review, the proposed drug substance specifications for all of the drug substance impurities exceed the threshold of toxicological concern of (b) (4) % (1.5 mcg/day) for genotoxic impurities. Specifically, (b) (4)

(b) (4) and (b) (4) (all with (b) (4) % specifications) contain structural alerts for mutagenicity. The alert moieties, however, are shared with the parent compound, neostigmine (see the table below for the structural comparison). Based on the structural similarities, and negative finding in the Ames test for the parent compound, the genotoxic potential of these impurities are considered to be negative based on the FDA Guidance (Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches, Draft Guidance, CDER 2008). Thus, the proposed impurity specifications are acceptable.

Chemical name	Structure
Neostigmine	

(b) (4)

(b) (4)

(b) (4)

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/s/  
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HUIQING HAO  
09/18/2012

RICHARD D MELLON  
09/18/2012  
I concur. See secondary review for further discussion.

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

**PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION**

Application number: 203629  
Supporting document/s: SDN-1  
Applicant's letter date: 12/28/2011  
CDER stamp date: 12/29/2011  
Product: Neostigmine Methylsulfate Injection  
Indication: Reversal agent to the neuromuscular blocking effects of non-depolarizing muscle relaxants  
Applicant: APP Pharmaceuticals LLC.  
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 A. Rappaport, M.D.  
Project Manager: Allison Meyer

*Template Version: September 1, 2010*

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# 1 Executive Summary

## 1.1 Introduction

Neostigmine is a cholinesterase inhibitor and the currently proposed product Neostigmine Methylsulfate Injection is indicated to reverse the neuromuscular blocking effects of non-depolarizing muscle relaxants.

Several neostigmine products were approved from 1939 through 1958 as eye drops, injection solution, and tablets for indications of glaucoma, myasthenia gravis, etc. The approved products were discontinued or withdrawn afterward with the latest withdrawal in 1995. However, as a reversal agent for non-depolarizing muscle relaxants, neostigmine has been continuously in the market without an approval and there are no approved neostigmine products currently. Based on an internet search, the Sponsor of this NDA, APP Pharmaceuticals LLC is one of the companies that currently market neostigmine products.

The Sponsor was informed in 2009 that based on the long history of clinical use no nonclinical studies for neostigmine drug substance would be required to support NDA approval. However, as no adequate data are available to allow appropriate labeling, genotoxicity and reproductive toxicology studies for neostigmine would be required as Post-Marketing-Requirements. Further, for NDA approval, studies may be required for novel excipients, leachables/extractables and impurities or degradants that exceed the threshold limits (preIND 106574, meeting minutes for meeting dated 12/22/2009).

## 1.2 Brief Discussion of Nonclinical Findings

There were no new nonclinical studies submitted. Based on published literature, toxicology information is summarized below:

Toxicities of neostigmine in animals are associated with excess nicotinic and muscarinic receptor activation. The toxic effects are marked 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 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 out from the circulation. Toxicities after repeated doses were similar to the acute toxicities but tolerance develops after a few doses.

The intravenous LD<sub>50</sub> values of neostigmine were 0.16 mg/kg in mice and 0.165 mg/kg in rats (Randall and Lehmann, 1950;Haley and Rhodes, 1950).

Genotoxicity and reproductive toxicology studies are not available in published literature and the Sponsor is required to complete these studies as Post-Marketing-Requirements.

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

### **1.3 Recommendations**

#### **1.3.1 Approvability**

Based on the structural alert and lack of mutagenicity study data for neostigmine and related impurities, this NDA is not recommended to be approved at this time.

#### Information Needed to Resolve Deficiencies

Conduct an in vitro bacterial reverse mutation assay with neostigmine.

#### **1.3.2 Additional Non-Clinical Recommendations**

There are no genotoxicity and reproductive toxicity data available. To allow adequate labeling, complete a standard battery of genotoxicity studies (except Ames test which is an approvability issue) and a standard battery of reproductive and developmental toxicology studies for neostigmine, as post marketing requirements.

#### **1.3.3 Labeling**

Note: sentences struck through indicate recommended deletions; sentences underlined indicate recommended additions. These recommendations have been prepared prior to review team discussion and should be deemed tentative. For final labeling recommendations, the reader is referred to the action letter.

(b) (4)



Note: It is not clear if the inhibitory effect of neostigmine on cholinesterase is reversible. There are no data demonstrating that the effect is reversible, rather irreversibility has been suggested in a rat study where examination of red blood cell acetylcholine esterase activity showed acetylcholinesterase inhibition persisted for several hours despite rapid clearance of neostigmine from blood (Barber, et al., 1979).

## 2 Drug Information

### 2.1 Drug

CAS Registry Number 51-60-5

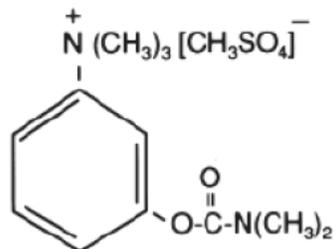
Generic Name Neostigmine methylsulfate

Code Name NA

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 Anticholinesterase inhibitor

### 2.2 Relevant INDs, NDAs, and DMFs

IND#	Drug	Status	Division	Indication	Stamp Date	Sponsor
106574	Neostigmine methylsulfonate	Presubmission	DAAAP	Reversal of Neuromuscular Blockade	9/15/2009	APP Pharmaceuticals LLC

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment

(b) (4)

### 2.3 Drug Formulation

Component	Content, per mL
Neostigmine methylsulfate, USP	1.0 mg or 0.5 mg
Phenol (liquefied, USP)	4.5 mg
Sodium acetate, USP (trihydrate)	0.2 mg
Water for injection, USP	Q.S. to 1 mL
(b) (4)	
(b) (4) acetic acid, USP	As needed
Sodium hydroxide, NF	As needed

### 2.4 Comments on Novel Excipients

The proposed product contains 4.5 mg/mL phenol as a preservative. This phenol concentration is acceptable as up to 5 mg/mL phenol has been used in FDA-approved intravenous products. Based on the proposed maximum daily dose of 5 mg neostigmine, and strength of 0.5 mg/mL and 1.0 mg/mL, the total dose of phenol would be 22.5 mg with the strength of 1.0 mg/mL (5 mL × 4.5 mg/mL) or 45 mg with the strength of 0.5 mg/mL (10 mL phenol × 4.5 mg/mL). There are no FDA-approved IV products associated with a single bolus dose of phenol up to this level (under NDA 19-667 for octreotide acetate, exposure to 15 mg phenol/day was given via three doses).

Phenol is toxic with probable oral human lethal dose of 50-500 mg/kg. Death and severe toxicity are usually due to effects on the CNS, heart, blood vessels, lung, and kidneys. Concentrated phenol is highly caustic to tissues. (Hazardous Substance Data Base, HSDB). ADME studies indicate that phenol is well absorbed by all route of administration (e.g., oral, inhalation and dermal exposure), widely distributed, and primarily eliminated in urine. Elimination half-life was reported in a range of 3.5-13.86

hours. This information, however, is inadequate to assess the toxicity associated with the proposed intravenous administration of phenol via the proposed drug product since the toxicity findings are based on occupational exposures to concentrated solutions.

In response to the Division's information request to justify the exposure to phenol in their drug product formulation, the Sponsor submitted their argument (June 4, 2012) and contended that the highest dose supported by literature for Neostigmine Methylsulfate Injection is 0.07 mg/kg. Using this dose, the maximum daily exposure for phenol is 44.1 mg/day for a 70 kg person. This dose level of phenol is lower than that given via approved products Tobramycin Injection, USP and Zantac Ranitidine Hydrochloride Injection. Based on the labeling, with severe cystic fibrosis, Tobramycin is recommended with an initial dose of 10 mg/kg/day in 4 equally divided doses. As Tobramycin Injection USP contains 40 mg of Tobramycin and 5 mg/mL of phenol, the daily dose of phenol would be 87.5 mg for a 70 kg person ( $10 \text{ mg/kg} \times 70 \text{ kg} \div 40 \text{ mg/mL} \times 5 \text{ mg/mL}$ ). Of note, due to pancreas-related issues severe cystic fibrosis patients are likely have lower body weights than general population and the 87.5 mg phenol exposure is likely overestimated. For Zantac® Ranitidine Hydrochloride Injection, with intermittent intravenous injection it may be necessary to increase dosage, but generally it should not exceed 400 mg/day in adults and 50 mg every 6 hours, or 200 mg/day in children. Each 1 mL of aqueous solution contains ranitidine 25 mg (as the hydrochloride); phenol 5 mg as a preservative. These dosing recommendations will results in the daily phenol dose of 80 mg/day for adults and 40 mg/day for children.

For the specific clinical conditions described above, phenol exposures were up to 87.5 mg/day via Tobramycin and 80 mg/day via Zantac. Thus, the proposed exposure of 45 mg phenol with the current product is supported in term of total daily dose. Note, the manner of administration differs as that the proposed dose of 45 mg (in 10 mL neostigmine) is given as a bolus injection whereas the referenced 87.5 mg in Tobramycin given as 4 doses (80 mg in Zantac given as 8 doses). The proposed phenol exposure would produce a 2-4 fold higher  $C_{\text{max}}$  (45 mg versus 87.5 mg/4 or 80 mg/8). The systemic and local toxicities associated with this  $C_{\text{max}}$  of phenol are not clear. Considering dose accumulation with repeated dosing of phenol based on an elimination half-life of 3.5 to 13.86 hours (HSDB), the difference on  $C_{\text{max}}$  probably is smaller than 2-4 fold (with half-life of 3.5 hours, dosing every four hours is estimated to produce 1.5x higher  $C_{\text{max}}$  after 4<sup>th</sup> dose compared to the initial dose). In light of the long history of clinical use of this product, the higher  $C_{\text{max}}$  is not predicted to present a significant concern; however, as this is dependent on clinical data, the reader is referred to the clinical review for a discussion of the clinical experience.

## 2.5 Comments on Impurities/Degradants of Concern

There are five impurities in drug substance were identified and will be controlled to the following specifications:

Impurity	Structure	Origin	Proposed Specification
(b) (4)			
Any Other Individual Impurity			NMT (b) (4) %
Total Impurity			NMT (b) (4) %

As per CMC reviewer, Dr. Edwin Jao, all five identified impurities contain a structural alert moiety. CDER's Computational Toxicology Group was requested to perform a QSAR analysis for mutagenicity via the Ames test. The analysis reported positive results for (b) (4) and predicted positive results for neostigmine, (b) (4) and inadequate information to predict potential for (b) (4) (Attachment 1).

Based on the maximal dose of 5 mg of neostigmine, and acceptance threshold of 1.5 mcg for genotoxic impurities, the acceptable levels for those impurities are NMT (b) (4) %. The specification for (b) (4) at (b) (4) % is acceptable. The specification of (b) (4) % for (b) (4) is also acceptable as (b) (4) based on extensive literature reports, assuming neostigmine itself is acceptable. All other impurities including (b) (4) and (b) (4) need to be qualified as the specifications exceed acceptance threshold of (b) (4) % for genotoxic impurities, and the proposed specifications are (b) (4)-fold higher ((b) (4) % versus (b) (4) %). Of note, the impurities either contain the exact same structural alert moiety or a

very similar moiety that triggered the concern of genotoxicity as neostigmine itself. If all are genotoxicity positive, the effect of impurities would be a miniscule compared that of neostigmine. Therefore, this (b) (4)-fold higher than acceptable impurity levels would not be an approvability issue. More importantly neostigmine genotoxicity should be studied and approvability should be reconsidered if positive.

The Division had a teleconference with the Sponsor on 4/13/2012 and discussed the Division's concerns for the potential genotoxicity of neostigmine and associated impurities, and urged the Sponsor to conduct Ames test for neostigmine and the impurities. On 4/20/2012, the Sponsor notified the Division that they planned to submit the Ames study in August of 2012 (email to Allison Meyer). As of the date of this review, the genetic toxicology study has not been submitted.

Drug product impurity/degradant specifications were provided as NMT (b) (4)% (b) (4) and NMT (b) (4)% other impurities. The degradant, (b) (4) at NMT (b) (4)% (b) (4) is acceptable based on acceptance threshold of 1.0% or 50 mcg TDI defined in ICH Q3B(R2) for drugs at daily dose less than 10 mg. The unidentified impurities/degradant at NMT (b) (4)% also meet the regulatory requirements described in ICH Q3B(R2).

There are no leachable and extractable profiles included in the NDA submission. Due to the concern of leachable profile produced in the presence of phenol that is contained in this product, the Division requested a leachable extractable study including a toxicological risk assessment of the identified leachables. Upon the Agency's requests (3/12/2012 of 74-Day letter and CMC request of 6/22/2012), the Sponsor responded that those profiles and safety justifications will be provided by August 31 of 2012 (submission dated July 30, 2012). During the course of the review, two other FDA-approved intravenous generic drug products were identified and these two products also contain (b) (4)% phenol and use the same (b) (4) stopper in their container closure system. Therefore, the lack of leachable profile is not an approval issue for this NDA at this time. The (b) (4) toxicological risk assessment submission expected on August 31, 2012 will be preliminarily reviewed to determine if the submitted data suggest a safety signal, and if need be, reviewed formally to support this action.

## 2.6 Proposed Clinical Population and Dosing Regimen

Neostigmine Methylsulfate Injection is indicated to reverse the neuromuscular blocking effects of non-depolarizing muscle relaxants. According to the Sponsor's proposed labeling, the recommended dose regimen is intravenous bolus injection at dose range of (b) (4)-0.07 mg/kg for both children and adults, titrated on an individual basis by monitoring of neuromuscular activities.

## 2.7 Regulatory Background

From 1939 to 1958, Hoffman-LaRoche Inc. received approvals for several neostigmine products (see the table below). These products, however, have been all discontinued with the latest one (NDA 654, ophthalmic solution) being withdrawn in 1995. There were no safety related discontinuations recorded.

N654 was withdrawn in 1995 due to not being marketed which was reported in 1988.

NDA No.	Product	Indication	Approval date	Outcome
654	Prostigmin Ophthalmic Solution (neostigmine bromide 5%)	glaucoma	3/27/1939	Withdrawn 7/27/1995, as no longer being marketed
2-449	Prostigmin and atropine (ampule for injection)	Intestinal peristalsis stimulant and myasthenia gravis and related disorders	5/9/1940	Discontinued in 1954, no further information recorded
2-574	Morphine-Prostigmin (hypodermic tablets for solution and injection)	Local anesthetic	6/4/1940	Dormant since approval and discontinued in 1948
2-575	Prostigmin and Pantopan (hypodermic tablet for solution and injection)	Narcotic analgesic	6/13/1940	Never marketed, withdrawn 3/28/1972

(b) (4)

Currently there are no FDA-approved neostigmine products in the market (Orange Book). However, several companies including General Injectables and Vaccines Inc., American Regent Inc., and Cardinal Health have been marketing unapproved neostigmine injection (<http://dailymed.nlm.nih.gov/dailymed/about.cfm>). Based on an internet search, App Pharmaceuticals Inc. and Baxter Pharmaceuticals also have been marketing neostigmine methylsulfate injection products.

### 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 has long been known as a cholinesterase inhibitor. By inhibiting acetylcholinesterase, neostigmine increases the acetylcholine (ACh) concentration in the neuromuscular junction in the synaptic cleft. For the proposed indication, the increased ACh more powerfully competes with nondepolarizing muscle relaxants for acetylcholine receptors (nicotinic receptors) to reverse the muscle relaxation.

In vivo, numerous studies reported that neostigmine reverses neuromuscular blockade produced by nondepolarizing muscle relaxants (pancuronium, vecuronium, atracurium, gallamine, alcuronium, d-tubocurarine, rocuronium and mivacurium) in rats, dogs, sheep and monkeys. In vitro, neostigmine inhibited red blood cell acetylcholinesterase with IC<sub>50</sub> of 6.9 nM (Harada, et al., 2010).

The major metabolite, 3-hydroxyphenyltrimethylammonium (HPTMA), was 6.1-fold less potent than neostigmine (ED<sub>50</sub> 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).

Alternative mechanisms of neostigmine effect were also studied. Neostigmine shortened a channel open time but did not affect the current amplitude in a single channel activated by 200 nM ACh. This effect was not secondary to inhibition of cholinesterase but appeared to involve a direct effect on acetylcholine-activated channel. The concentration that reduced 50% of channel open time was 4.6 mcM

neostigmine, which was reported to be higher than clinically encountered (Wachtel, 1990).

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

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).

Other secondary pharmacological effects of neostigmine were also studied including changes of glucose regulation by intracerebroventricular or hypothalamic/hippocampal administration, antinociceptive effects by intrathecal injection with neostigmine alone or in combination with NSAIDs or morphine, induction of sleep by injection into pontine reticular formation (PRF), etc. As these studies are unrelated to the indication of this NDA, no review is rendered here.

## 4.3 Safety Pharmacology

CNS effects:

As neostigmine does not cross blood-brain barrier, no 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 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:

On respiratory system, 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 mediated by peripheral

nicotinic receptors 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 a 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

Orally administered neostigmine is poorly absorbed. In rats given <sup>14</sup>C-labeled neostigmine 250 mcg orally resulted in approximately 50% of dose detected in the intestinal content and feces, and about 20% of dose excreted in the urine by 24 hours postdosing (Roberts, et al., 1966).

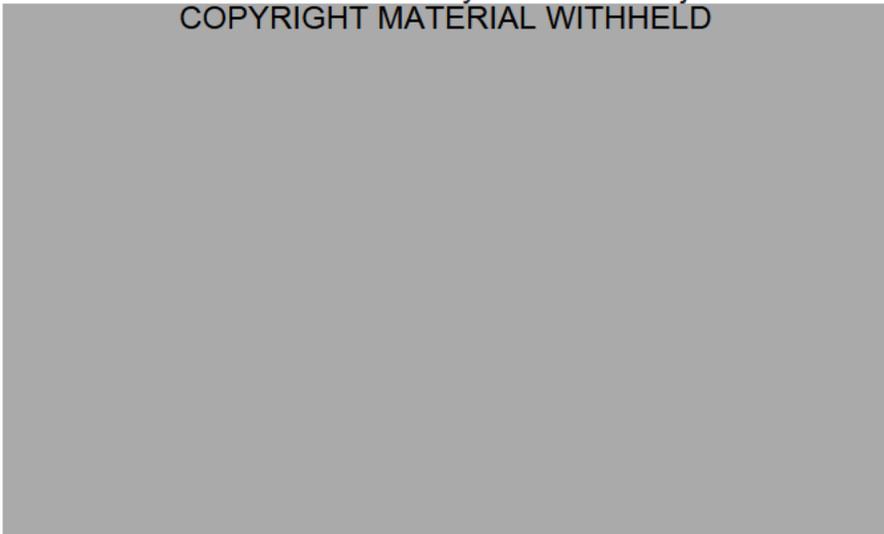
Tissue distribution of neostigmine was found in the liver, muscle, heart and kidney. Following a single subcutaneous administration of <sup>14</sup>C-neostigmine (1.68 mcmol/kg) to rats, T<sub>1/2</sub> was 10 minutes in plasma, 33 minutes in liver and 1.5 hours in muscle. Levels of radioactivity in 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 <sup>14</sup>C-neostigmine iodide, plasma radioactivity rapidly disappeared, with 1% remained by 120 minutes post injection. At this time point, <sup>14</sup>C 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 pass 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-minute 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 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 NADPH<sub>2</sub> (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).

Following oral administration of  $^{14}\text{C}$ -neostigmine, HPTMA accounts for 90% of the radioactivity of urine in rats (Roberts, et al., 1966). Urine elimination as unchanged neostigmine was a more significant portion following parenteral administration compared to oral administration. In rats given 25 mcg of  $^{14}\text{C}$ -neostigmine intramuscularly, about 30% of the dose was 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, as noted in the figure below reproduced from the published literature (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 turbocurarine-induced tibial muscle twitch depression (Miller and Roderick, 1977). Thus, based on animal data, renal excretion of parent drug plays a role in elimination of neostigmine given parenterally and doses given to patients with severe renal disease may need to be adjusted.

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

Neostigmine (+), PTMA ( $\times$ ), PTMA-glucuronide ( $\bullet$ ) (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 toxicokinetics data available.

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

Published literature reported that toxicities of neostigmine are 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 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 out from the circulation.

The intravenous LD<sub>50</sub> values of neostigmine were 0.16 mg/kg in mice and 0.165 mg/kg in rats (Randall and Lehmann, 1950;Haley and Rhodes, 1950).

A study showed that neostigmine has more muscarinic effects (incidence/severity of salivation and airway secretion, defecation and flatulence) than edrophonium (Hildebrand and Howitt 1984). Dogs given 0.035 mg/kg neostigmine intravenously showed intraluminal colonic pressure rose 15 times above baseline, accompanied with severe spasm, associated with foreshortening and hypersegmentation of the bowel (Yellin, et al., 1973).

Additional clinical signs including increased glucose, seizures, behavioral changes, fasciculations, motor weakness, tremors, rigidity, urination, and miosis were observed when neostigmine was administered by the intrathecal or intracerebroventricular routes, or by injection into the hypothalamus or hippocampus. Moderate synovial membrane cell hypertrophy was reported in rabbits given intra-articular injection of neostigmine (0.05 mg/0.25 mL). Those findings are not considered clinically relevant to this NDA due to lack of significant drug exposures to these site when administered intravenously as proposed.

### 6.2 Repeat-Dose Toxicity

Similar to acute toxicities were observed in early phase of repeat-dose studies, but tolerance seems to develop to neostigmine effects after repeated dosing.

Rats given 0.1 mg neostigmine subcutaneously twice daily for 3 days or 22-25 days. Generalized tremor, muscle fasciculation, ruffling of the fur, excessive salivation, tachypnea, decreased voluntary activity, and apparent weakness were observed at 2-4 minutes after a single a single subcutaneous injection of 0.1 mg of neostigmine methylsulfate. There 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).

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

## **7 Genetic Toxicology**

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

No studies available

### **7.2 *In Vitro* Assays in Mammalian Cells**

No studies available

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

No studies available

### **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. In order to appropriately inform drug product labeling, these studies should be completed as post-marketing requirements.

## 10 Special Toxicology Studies

None

## 11 Integrated Summary and Safety Evaluation

### Introduction

Currently there are no approved neostigmine products in the market. However, the proposed product Neostigmine Methylsulfate Injection has been an unapproved product in the market for decades. Based on the long history of clinical use, nonclinical studies for neostigmine were not required to support the NDA. The following nonclinical information was provided based on published literature.

### Pharmacology

Neostigmine is a cholinesterase inhibitor indicated to reverse neuromuscular blocking effects of non-depolarizing neuromuscular blocking agents. By reducing the hydrolysis of acetylcholine, neostigmine increases the synaptic levels of acetylcholine and 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 cross blood-brain-barrier. Following an intravenous dose, neostigmine is rapidly cleared from plasma, metabolized in the liver and excreted in urine in the forms including unchanged parent compound, major metabolite 3-hydroxyphenyltrimethylammonium (HPTMA) and 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 indicates the potential need of dose adjustment for patients with severe renal function impairment. The elimination T<sub>1/2</sub> is in a range of 7.3-23.5 minutes in the rat, dog and guinea pig and 53 minutes in the human.

### Toxicology

Toxicities of neostigmine in animals are 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 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 out from the circulation. Toxicities after repeated doses were similar to the acute toxicities but tolerance does appear to develop after a few doses.

The intravenous LD<sub>50</sub> values of neostigmine were 0.16 mg/kg in mice and 0.165 mg/kg in rats (Randall and Lehmann, 1950;Haley and Rhodes, 1950).

There are no genotoxicity and reproductive toxicology studies available. Based on the long history of human use, the Division has informed the Sponsor that such studies will not be required for approval but would likely be required to be completed as Post-Marketing Requirements (meeting minutes for preIND106754 meeting dated 12/22/2009). However, upon review of the drug substance impurities, it was noted that neostigmine and the drug substance impurities contain structural alerts for genotoxicity which are predicted to be genotoxic based on a computational toxicology analysis. As such, based on these new data, I recommend that a genetic toxicology study for neostigmine be completed prior to approval to support the safety of neostigmine and the related impurities. We discussed this concern with the Sponsor during the review cycle and encouraged them to conduct an in vitro bacterial mutation assay. The Sponsor plans to submit the data by 8/31/2012. If neostigmine is positive in the Ames assay, then the risk:benefit of this drug product will have to be considered.

Additionally, there are no carcinogenicity data available. However, based on the proposed indication of acute use, carcinogenicity studies are not necessary at this time. Should the standard battery of genetic toxicology studies demonstrate a cause for concern, this will have to be re-evaluated.

#### 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 in other FDA-approved intravenous drug products at comparable levels and do not raise safety concerns. The proposed specification of (b) (4) % phenol would produce up to (b) (4) mg bolus exposure via the maximum human dose of neostigmine (5 mg). The concentration of (b) (4) % and total daily dose of (b) (4) mg are within the range of approved uses of phenol for intravenous administrations. However, the proposed bolus dose of (b) (4) 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. We have been unable to identify any IV toxicology study that would have characterized the potential toxicities of this bolus infusion. We acknowledge that there is extensive clinical experience with this marketed-unapproved drug product; therefore, this magnitude of higher  $C_{max}$  has been tested in humans. The safety of this bolus dose of phenol may be justified based on clinical data, at the discretion of the medical team. .

Leachables/extractables and impurities or degradants: There was no leachable/extractable profile provided. This deficiency was communicated to the Sponsor early in the review cycle and the studies were initiated. (b) (4)

Drug substance impurities: There are five impurities in drug substance including (b) (4). All of these impurities contain a structural alert moiety, most of which are also found in the parent compound neostigmine. Among those impurities, proposed specifications for (b) (4)

(b) (4) and (b) (4) are acceptable based on acceptable threshold of 1.5 mcg/day for (b) (4) (b) (4) (b) (4). All other impurities were specified to be NMT (b) (4) which is higher than acceptable threshold of 0.03% (1.5 mcg/day based on MRHD of 5 mg neostigmine). The Sponsor agreed to generate genotoxicity data to attempt to address these concerns. The Agency was informed that these studies would be submitted to the NDA in August; however, to date, no study reports have been submitted.

Drug product degradants: The proposed specifications including NMT (b) (4)% (b) (4) and NMT (b) (4)% other impurities are acceptable as they all meet acceptable 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  
**Re:** NDA 203629  
**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), Leadscape 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.

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(b) (4)



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<sup>1</sup> + = 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; I = test chemical is experimentally inactive

Page 1



(b) (4)

This report has been reviewed and approved by CDER/OPS/OTR/DDSR.

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/s/  
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HUIQING HAO  
08/28/2012

RICHARD D MELLON  
08/28/2012

I concur with the recommendation of a complete response at this time. Please see secondary review for further discussion.

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

**NDA/BLA Number: 203-629**

**Applicant: APP  
Pharmaceuticals**

**Stamp Date: Dec. 28, 2011**

**Drug Name: Neostigmine**

**NDA/BLA Type: 505(b)2**

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
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)?	√		
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).		√	APP Pharmaceuticals has been marketing the product without approval. 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/BLA or Supplement**

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
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?	√		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m <sup>2</sup> 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 provided [REDACTED] (b) (4)
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

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

As per the 1999 FDA Guidance for Industry titled “Container Closure Systems for Packaging Human Drugs and Biologics”, injectable drug product container closures present the highest degree of concern regarding the likelihood of potentially leaching harmful substances into the drug product solution. Although you have submitted results of USP testing for the [REDACTED] (b) (4) gray [REDACTED] (b) (4) rubber stopper, your submission does not appear to include an extraction study to determine which chemical species may migrate into the dosage form (and at what concentration) or a toxicological evaluation of those specific substances which are extracted to justify the safe level of exposure via this drug product. As noted in the guidance, data from USP Biological Reactivity Tests and USP Elastomeric Closures for Injections tests are typically considered sufficient evidence of material safety; however, given the presence of phenol in your drug product solution, we are not convinced that potentially novel compounds (chemical identify or concentration) will not leach from this stopper. Submit

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement 010908

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

data from controlled extraction studies to qualitatively and quantitatively determine the chemical species which may migrate into the dosage form, and leachable data from long-term stability studies (taking into consideration the proposed shelf-life) to determine if the identified/specified extractables also leach into the drug product over time, and a toxicological risk assessment justifying the safety of the extractables and leachables taking into consideration the maximum daily dose of the identified materials for this drug product.

Huiqing Hao, Ph.D.	Jan. 24, 2012
_____ Reviewing Pharmacologist	_____ Date
Dan Mellon, Ph.D.	Jan 25, 2012
_____ Team Leader/Supervisor	_____ Date

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/s/  
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HUIQING HAO  
01/25/2012

RICHARD D MELLON  
01/25/2012