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Acute Oral Toxicity of Physostigmine
Salicylate in Guinea Pigs

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and
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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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ABSTRACT

The acute oral toxicity of physostigmine salicylate was determined in male and female Hartley guinea pigs using the single-dose method. The median lethal dose for both male and female guinea pigs was less than 7.1 mg/kg. Clinical signs observed were primarily related to changes in behavior; such as tremors, irritability, ataxia, and inactivity. Other frequently observed clinical signs included salivation, diarrhea, and lacrimation. The duration of the clinical signs was acute. Most animals were exhibiting signs by 24 hours after dosing and had either died or returned to normal by 72 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place physostigmine salicylate in the highly toxic class.

Key Words: Acute Oral Toxicity, Physostigmine Salicylate, Guinea Pig, RA V



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PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

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Letterman Army Institute of Research
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SPONSOR:

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US Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5425
Project Officer: LTC J. von Bredow, PhD, MSC

PROJECT/WORK UNIT/APC: 3M162734A875/308/TLE0

GLP STUDY NUMBER: 87008

STUDY DIRECTOR: Don W. Korte, Jr., PhD, MAJ, MS

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DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT: A copy of the final report,
study protocols, retired SOPs,
raw data, analytical, stability,
and purity data of the test
compound, and an aliquot of the
test compound will be retained in
the LAIR Archives.

TEST SUBSTANCE: Physostigmine Salicylate

INCLUSIVE STUDY DATES: 7 July - 24 September 1987

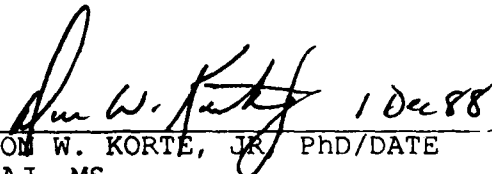
OBJECTIVE: The objective of this study was to determine the
acute oral toxicity of physostigmine salicylate in male
and female Hartley guinea pigs.


ACKNOWLEDGMENTS

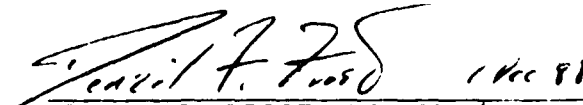
SPC Dean Magnuson, BS, and SPC Joel B. Seewald, BS, provided research assistance; SGT John R. G. Ryabik, BS, provided chemical preparation and analysis; SGT Tammie R. Heineman, SGT Chuck Freedman, and Richard A. Spieler provided animal care and facility management; and Marie Rogers and Mara Joshua provided secretarial assistance.

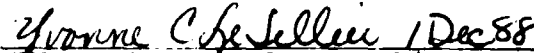
**SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS
INVOLVED IN THE STUDY**


We, the undersigned, declare that study number 87008 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.


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DEPARTMENT OF THE ARMY

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REPLY TO
ATTENTION OF

SGRD-ULZ-QA

30 November 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 87008

1. This is to certify that in relation to LAIR GLP Study 87008, the following inspections were made:

| | |
|-------------------|-------------------------------------|
| 06 April 1987 | - Protocol Review |
| 23 July 1987 | - Dosing and Observations (Phase I) |
| 04 August 1987 | - Final Sacrifice (Phase I) |
| 08 September 1987 | - Dosing (Phase II) |
| 08 September 1987 | - Observations (Phase II) |
| 22 September 1987 | - Final Observations (Phase II) |
| 22 September 1987 | - Final Sacrifice (Phase II) |

2. The institute report entitled "Acute Oral Toxicity of Physostigmine Salicylate in Guinea Pigs," Toxicology Series 217, was audited on 14 November 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

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Acute Oral Toxicity of Physostigmine Salicylate in Guinea Pigs -- Frost and Korte

INTRODUCTION

Soman, the primary nerve agent utilized by threat forces, is refractory to the standard antidotal therapy, atropine and pralidoxime (2-PAM), fielded by the US Army. Consequently, the highest priority has been placed on fielding a more effective treatment regimen. A regimen incorporating pyridostigmine as a prophylactic agent, combined with standard atropine/2-PAM therapy, has proven extremely effective in reducing mortality of Rhesus monkeys to multilethal concentrations of soman (1). However, these animals require a prolonged period of recovery during which they are completely incapacitated. This has been attributed to the quaternary nature of pyridostigmine, which does not cross the blood-brain barrier and thus only protects the peripheral nervous system. Consequently, a tertiary carbamate, physostigmine, has been proposed for the prophylactic regimen since it would protect the central nervous system in addition to the peripheral nervous system. Experimental studies support this hypothesis as animals pretreated with physostigmine before exposure to soman recover at a faster rate than animals pretreated with pyridostigmine (2,3). An enhanced rate of recovery of soldiers from a multilethal exposure to soman would produce a decided advantage in maintaining a fully functional military unit during a future conflict.

The only approved formulation of physostigmine is for intravenous administration, which is not a feasible option for the proposed prophylactic therapy. Either the oral or dermal route of administration for prophylactic therapy would be feasible. However, even though physostigmine has been available for more than a century (4), little directed research on its toxicology following oral or dermal administration has been conducted. Consequently, the Division of Toxicology, Letterman Army Institute of Research, was tasked by the US Army Medical Research Institute of Chemical Defense to provide an acute and subchronic toxicity profile of physostigmine salicylate following oral and subcutaneous administration.

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Objective of Study

The objective of this study was to determine the acute oral toxicity of physostigmine salicylate in male and female Hartley guinea pigs.

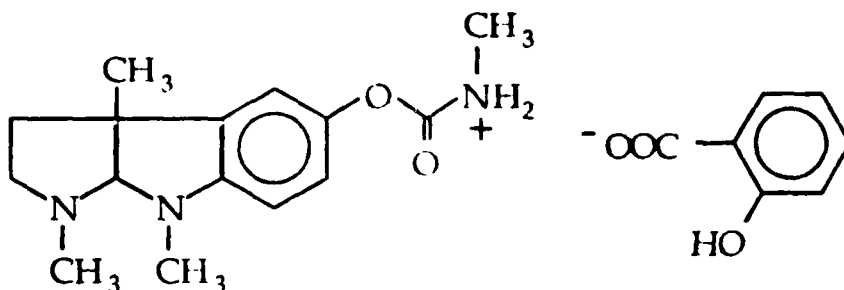
MATERIALS

Test Substance

Chemical Name: Physostigmine salicylate

Chemical Abstracts Service Registry No.: 57-64-7

Chemical Structure:



Molecular Formula: C₁₅H₂₁N₃O₂ • C₇H₆O₃

Source: Mr. William Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Requested by LTC J. von Bredow, USAMRICD

Other test substance information is presented in Appendix A.

Vehicle

The vehicle for physostigmine salicylate was sterile water (Abbott Labs, North Chicago, IL 60064). The expiration date was 1 February 1989, and the lot number was 01-075-FW.

Animal Data

Forty-seven male and 87 female Hartley guinea pigs (Charles River Laboratories, Inc., Kingston, NY) were used for this study. They were identified individually with ear tags. Two additional males and three females from the shipment were

randomly selected for quality control necropsy. The animal weights on receipt (8 Jul 87, 28 Aug 87) ranged from 188 g to 265 g. Additional animal data appear in Appendix B.

Husbandry

Guinea pigs were caged individually in stainless steel wire-mesh cages with automatic flushing dumptanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow[®] Diet 5026 (Ralston Purina Company, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 17.7°C to 25.6°C with a relative humidity range of 38% to 67%. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study animals were randomized into 5 dose groups of 8 males each, 4 dose groups of 8 females each, 3 dose groups of 16 females each, and vehicle control groups of 5 males and 5 females each. Allocation was accomplished using a computer-based, stratified, weight-biased method. The Beckman TOXSYS[®] Animal Allocation Program was used in conjunction with a Beckman TOXSYS[®] Data Collection Terminal. The animals were acclimated for 12-19 days before the day of dosing. During this period they were observed daily for signs of illness.

Dosage Levels

The ALD determination indicated that the median lethal dose (MLD) was between 5 and 7.5 mg/kg. Based on these data, test dosages were selected (Table 1).

Compound Preparation

Specific concentrations for dosing were prepared in sterile water for injection (Abbott Laboratories, North Chicago, IL 60064, Lot No. 01-075-FW).

TABLE 1: Physostigmine Salicylate Dosages

| Group | Dosage Level (mg/kg) |
|------------------------------------|-------------------------|
| Phase I (males and females) | |
| 1 | 5.62 |
| 2 | 6.31 |
| 3 | 6.65 |
| 4 | 7.08 |
| 5 | 7.50 |
| 6 (vehicle control) | - |
| Phase II (females only) | |
| 1 | 5.62 |
| 3 | 6.65 |
| 5 | 7.50 |
| 7 | 4.47 |
| 8 | 8.91 |

Chemical Analysis of Dosing Solution

The concentration of physostigmine salicylate in the dosing solutions was determined by UV spectrophotometry (Appendix A). Actual concentrations of physostigmine salicylate in the dosing solutions ranged from 96.8% to 99.6% of the target concentration.

Test Procedures

This study was conducted in accordance with LAIR SOP OP-STX-36 (5).

The volume of dosing solution each animal received was based upon the desired dose level, the compound's concentration in suspension, and the animal's weight. Volumes ranged from 2.1 ml to 3.5 ml in the males and from 2.4 ml to 3.8 ml in females. The vehicle control group was given 2.7 ml to 3.3 ml sterile water. The volumes given were based on 10 ml/kg. Dosing was performed using the oral gavage method without animal sedation or anesthesia. Sterile disposable syringes (Becton, Dickerson & Co, Rutherford, NJ) fitted with 5-French feeding tubes (Seamless Hospital Products Co., Division of Dart Industries, Inc., P.O. Box 828, Wallingford, CN) were used for dosing. Phase I animals were dosed between 0745 and 1336 hours on 21 Jul 87 and 0737 and 0903 on 23 Jul 87; Phase II animals were dosed between 0943 and 1020 hours on 8 Sep 87, 0921 and 0939 on 10 Sep 87, and 0900 and 0919 on 15 Sep 87.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages; (b) animals were removed from their cages and given a physical examination; and (c) animals were observed after being returned to their cages. On the day of the dosing, the animals were checked intermittently throughout the day. Recorded observations were performed 1, 2, and 4 hours after dosing, and daily for the remainder of the 2 week test period. A second "walk-through" observation was performed daily and only significant observations recorded. Body weights were recorded once weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those which survived the 14-day study period were submitted for necropsy immediately after sacrifice by barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The LD10, LD50, and LD90 were derived by the maximum likelihood method of probit analysis, as described by Finney (6). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to plot the probit curve and lethal dose values.

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Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

The dosing phase of this study was accomplished according to the protocol and applicable amendments with the following exceptions: The dosage levels for Phase I and II were differentiated between males and females to allow a more accurate MLD determination for each sex, and pediatric feeding tubes were used in place of metal dosing needles to minimize potential trauma to the oral and esophageal mucosa. It is believed that none of these changes had any adverse effect on the results of this study.

Storage of Raw Data and Final Report

A copy of the final report, study protocol, raw data, retired SOPs and an aliquot of the test compound will be retained in the Letterman Army Institute of Research Archives.

RESULTS

Mortality

Sixty-three animals died as a result of the dosing. Fifty-one (81%) deaths occurred within 12 hours of dosing. An additional 11 (17%) deaths occurred by 48 hours after dosing. Table 2 lists the compound-related deaths by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis, and the equation for the probit regression line was:
 $Y = -0.47 + 6.44 \log X$ (males) and $Y = 0.66 + 5.61 \log X$ (females), where X is the dose and Y the corresponding probit value. Lethal doses calculated from the equation for the probit regression line are presented in Table 3. Figures 1 and 2 graphically present the actual data points and the regression line.

TABLE 2: Compound-Related Deaths by Group

| Group | Dose Level (mg/kg) | Compound Related Death/ Number in Group | Percent Mortality |
|----------------|-----------------------|--|----------------------|
| MALES | | | |
| 1 | 5.62 | 3/ 8 | 38 |
| 2 | 6.31 | 1/ 8 | 13 |
| 3 | 6.65 | 4/ 8 | 50 |
| 4 | 7.08 | 4/ 8 | 50 |
| 5 | 7.50 | 5/ 8 | 63 |
| 6 | Control | 0/ 5 | 0 |
| FEMALES | | | |
| 7 | 4.47 | 2/ 8 | 25 |
| 1 | 5.62 | 8/16 | 50 |
| 2 | 6.31 | 5/ 8 | 63 |
| 3 | 6.65 | 9/16 | 56 |
| 4 | 7.08 | 5/ 8 | 63 |
| 5 | 7.50 | 9/15 | 60 |
| 8 | 8.91 | 8/ 8 | 100 |
| 6 | Control | 0/ 5 | 0 |

Clinical Observations

Fifty-one out of 129 dosed animals died within 12 hours of dosing. Clinical signs, in many cases, were observed before death. Many of the surviving 79 dosed animals exhibited clinical signs within the first 24 hours after dosing. The clinical observations included gastrointestinal, behavioral, reflexive, skin/fur, urogenital, and ocular signs.

TABLE 3: Calculated Lethal Doses (LD) of Physostigmine Salicylate in Sprague-Dawley Rats

| Level | Calculated Dose* (mg/kg) |
|----------------|-----------------------------|
| MALES | |
| LD10 | 4.48 ± 1.35 |
| LD50 | 7.08 ± 0.62 |
| LD90 | 22.0 ± 2.57 |
| FEMALES | |
| LD10 | 3.51 ± 0.78 |
| LD50 | 5.94 ± 0.40 |
| LD90 | 10.05 ± 1.66 |

* Calculated dose ± standard error

The most frequently observed category of signs was gastrointestinal disturbances (72 of 129 animals). Gastrointestinal signs exhibited by the animals included salivation, diarrhea, tarry feces, stain or material found on animal, and emaciation. Gastrointestinal signs were present in all dose groups but did not exhibit a dose-response relationship. Other classes of clinical signs observed and their frequencies included: behavioral (15/129), reflexive (1/129), skin/fur (7/129), ocular (1/129), and urogenital (1/129). Of interest was one prolapsed uterus 2 days after dosing (animal 87E00261).

Most clinical signs had cleared by 72 hours after dosing, with the exception of diarrhea, inactivity, staining, pallor, tarry feces, rough coat, and emaciation. Twenty-five animals died within the first 48 hours without clinical signs being detected. Additional deaths with clinical signs observed occurred up to 4 days after dosing. Tables 4 and 5 contain a summary of clinical observations. Appendix E contains individual animal histories.

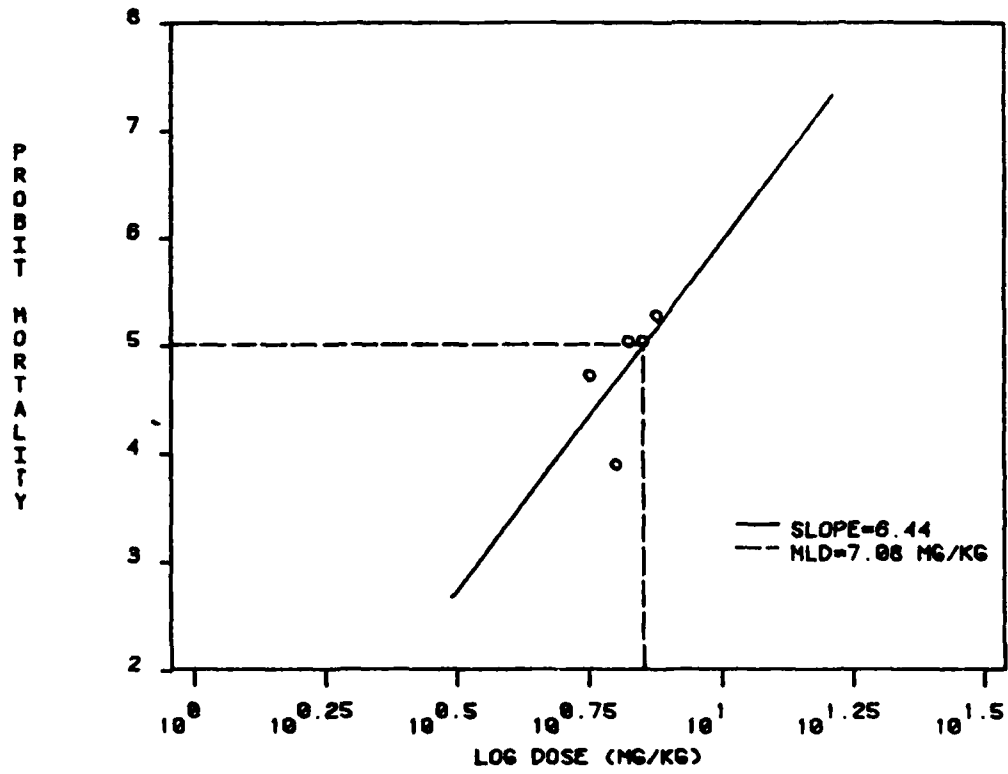


Figure 1: Physostigmine Dose Response Curve in Male Guinea Pigs

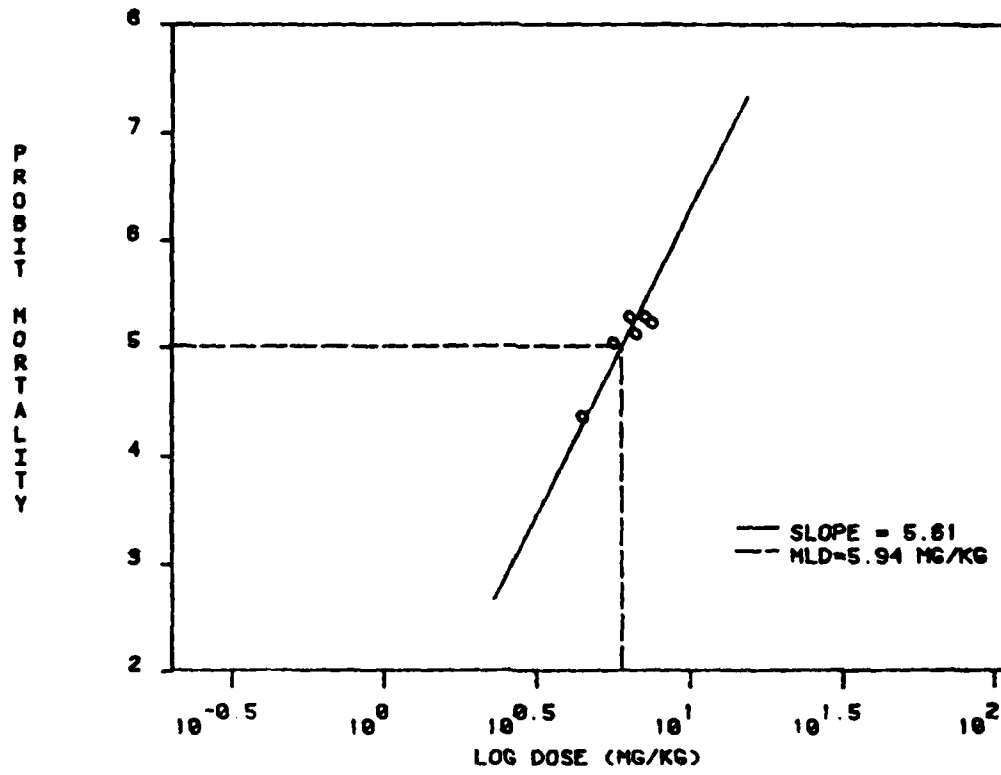


Figure 2: Physostigmine Dose Response Curve in Female Guinea Pigs

TABLE 4
 Incidence Summary for Clinical Observations in Male Guinea Pigs
 Administered Physostigmine Salicylate

| Clinical Signs | Dose (mg/kg) | | | | | | Vehicle |
|-------------------------------|--------------|------|------|------|------|---|---------|
| | 5.62 | 6.31 | 6.65 | 7.08 | 7.50 | | |
| | 8 | 8 | 8 | 8 | 8 | 5 | |
| Behavior ^a | - | 1 | - | - | 3 | - | |
| Gastrointestinal ^b | 5 | 5 | 3 | 3 | 3 | 1 | |
| Skin/fur ^c | - | 1 | - | - | 1 | - | |

^aIncludes inactive and tremors.

^bIncludes increased salivation, diarrhea, tarry feces, stains or material found on animal, and emaciation.

^cIncludes rough coat.

TABLE 5
Incidence Summary for Clinical Observations in Female Guinea Pigs
Administered Physostigmine Salicylate

| Clinical Signs | Dose (mg/kg) | | | | | | | | | |
|-------------------------------|--------------|------|------|------|------|------|------|---------|--|--|
| | 4.47 | 5.62 | 6.31 | 6.65 | 7.08 | 7.50 | 8.91 | Vehicle | | |
| | 8 | 16 | 8 | 16 | 8 | 15 | 8 | 5 | | |
| General ^a | - | - | - | 1 | - | - | - | - | | |
| Behavior ^b | 1 | - | 1 | 2 | - | 6 | 1 | - | | |
| Reflexes ^c | 1 | - | - | - | - | - | - | - | | |
| Ocular ^d | - | - | - | - | - | 1 | - | - | | |
| Gastrointestinal ^e | 7 | 9 | 5 | 9 | 5 | 9 | 6 | 2 | | |
| Skin/fur ^f | 2 | 1 | 1 | 2 | - | - | - | - | | |
| Urogenital ^g | - | 1 | - | - | - | - | - | - | | |

^aIncludes hunched posture.
^bIncludes inactive, ataxia, paralysis, and tremors.
^cIncludes loss of equilibrium.
^dIncludes lacrimation.
^eIncludes increased salivation, diarrhea, tarry feces, stains or material found on animal, and emaciation.
^fIncludes pallor and rough coat.
^gIncludes prolapsed uterus.

Weight gains of survivors were not significantly affected by dosing. Tables 6(a) and 6(b) present the mean body weights by groups. Appendix F contains individual weight tables.

Pathology Findings

There were no lesions observed at necropsy in those animals euthanized after the 14-day observation period. The majority of animals that died during the study presented with a serous oral discharge, perianal staining, and intussusception, observations consistent with the actions of a parasympathomimetic agent. Appendix G contains the complete pathology report.

DISCUSSION

The acute oral administration of physostigmine salicylate to Hartley guinea pigs produced pronounced toxicological effects. The calculated median lethal dose (MLD) for physostigmine salicylate in this study was 7.08 mg/kg in male and 5.94 mg/kg in female guinea pigs. These MLD values place physostigmine in the highly toxic classification (1-50 mg/kg) of Hodge and Sterner (7).

The toxicity observed following physostigmine administration was consistent with massive cholinergic stimulation following cholinesterase inhibition (8). Toxic signs attributable to excessive muscarinic stimulation included lacrimation, salivation, and diarrhea. The nicotinic effects observed included tremors, irritability, inactivity, and ataxia as the animals became fatigued. These effects were observed primarily in surviving animals since many animals that received the higher doses died without exhibiting the spectrum of toxic signs observed in animals receiving the lower doses.

The two cases of ileocolic intussusceptions observed in animals 87E00239 and 87E00257 at necropsy is probably related to the acetylcholine release that occurs following physostigmine salicylate administration (8). The prolonged toxicity and late death observed in one female (87E00228) administered 6.31 mg/kg physostigmine salicylate was not expected since the half-life of physostigmine is short in all species tested (9). The mechanism for this delayed toxicity cannot be elucidated from this study. If the delayed toxicity is due to a direct pharmacological effect, it will become readily apparent in subchronic toxicity studies scheduled to be conducted in several species.

**TABLE 6(a): Mean Body Weights of Male Guinea Pigs
Administered Physostigmine Salicylate**

| Dose Groups (mg/kg) | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|------------------------|--------------------------------|--------------------|--------------------|------------------------|
| 5.62 | 235.1 [†] ±4.6 (8) | 303.0 ±7.7 (8) | 349.6 ±23.5 (5) | 360.6 ±19.9 (5) |
| 6.31 | 230.1 ±6.2 (8) | 300.1 ±5.2 (8) | 335.7 ±20.3 (7) | 347.0 ±25.1 (7) |
| 6.65 | 228.1 ±7.4 (8) | 304.5 ±14.7 (8) | 375.5 ±18.1 (4) | 391.0 ±20.2 (4) |
| 7.08 | 228.9 ±5.7 (8) | 304.0 ±7.1 (8) | 349.3 ±6.5 (4) | 363.5 ±9.7 (4) |
| 7.50 | 228.9 ±6.3 (8) | 301.4 ±8.6 (8) | 359.3 ±21.2 (3) | 366.3 ±27.7 (3) |
| Vehicle Control | 229.4 ±3.2 (5) | 305.0 ±8.9 (5) | 371.6 ±10.8 (5) | 380.2 ±16.1 (5) |

*Weight after overnight fast.

[†]Values are mean ± standard error (number of animals) in grams.

TABLE 6(b): Mean Body Weights of Female Guinea Pigs Administered Physostigmine Salicylate

| Dose Groups (mg/kg) | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|------------------------|--------------------|--------------------|--------------------|------------------------|
| 4.47 | 235.0† ±8.8 (8) | 293.8 ±14.2 (8) | 317.7 ±20.4 (6) | 313.3 ±26.9 (6) |
| 5.62 | 233.2 ±4.8 (16) | 287.5 ±8.4 (16) | 324.8 ±15.4 (8) | 327.1 ±14.9 (8) |
| 6.31 | 230.1 ±5.6 (8) | 273.4 ±8.9 (8) | 328.0 ±9.8 (3) | 334.3 ±10.5 (3) |
| 6.65 | 235.1 ±6.6 (16) | 289.7 ±4.4 (16) | 340.9 ±10.2 (7) | 349.1 ±10.6 (6) |
| 7.08 | 236.3 ±3.8 (8) | 287.6 ±3.9 (8) | 340.7 ±5.7 (3) | 333.7 ±10.7 (3) |
| 7.50 | 242.9 ±4.3 (15) | 278.1 ±6.4 (15) | 329.5 ±16.4 (6) | 335.2 ±16.0 (6) |
| 8.91 | 252.8 ±8.8 (8) | 342.4 ±9.3 (8) | - | - |
| Vehicle Control | 216.8 ±7.2 (5) | 276.8 ±6.0 (5) | 329.4 ±3.6 (5) | 329.2 ±3.7 (5) |

*Weight after overnight fast.

†Values are mean ± standard error (number of animals) in grams.

CONCLUSION

Physostigmine salicylate is a highly toxic compound that produces clinical signs associated with cholinergic stimulation. Calculated MLD values after oral administration were 7.08 mg/kg in male and 5.94 mg/kg in female Hartley guinea pigs.

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APPENDICES

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APPENDIX A: Chemical Data

Chemical Name: Physostigmine salicylate

Other Names: Eserine salicylate; Physostigmine, 2-hydroxybenzoate; 1, 2, 3, 3a, 8, 8a-Hexahydro-1, 3a, 8-trimethylpyrrolo[2,3-b]indol-5-ol methylcarbamate (ester), (3aS-cis)-, mono (2-hydroxybenzoate) (salt)

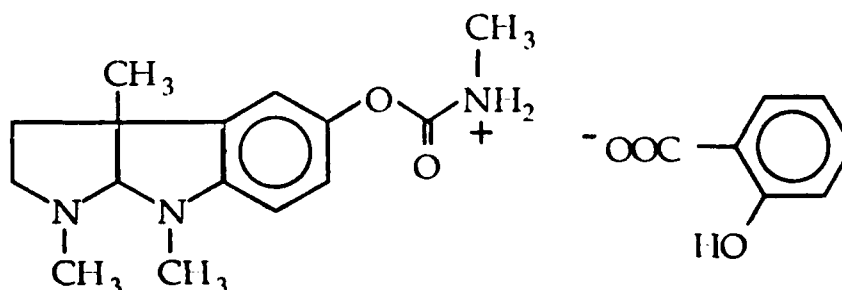
Lot Number: BL25591

Chemical Abstracts Service Registry Number: 57-64-7

LAIR Code: TW73

WRAIR Code: WR 6570AM

Chemical Structure:



Molecular Formula: $C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$

Molecular Weight: 413.47

Analytical Data:

The test compound was analyzed by the sponsors and the identity confirmed by UV and IR spectroscopy, high pressure liquid chromatography, mass spectrometry and elemental analysis.¹ Based on HPLC analysis of this test compound in comparison with the USP physostigmine salicylate reference standard, lot BL25591 contains 66.7% (100.1% of theory) physostigmine base and 33.7% (100.8% of theory) salicylic acid or 100.4% physostigmine salicylate.¹

HPLC analysis of physostigmine salicylate in this lab was performed using a Hewlett-Packard 1090 HPLC system equipped with a diode array detector. The compound was chromatographed under the following conditions: silica

APPENDIX A (cont.): Chemical Data

column (4.6 x 100 mm, Brownlee Labs, Inc.); mobile phase, 15% acetonitrile/buffer (0.01M Na₂HPO₄ with 0.0025M tetramethylammonium chloride); flow rate, 1.5 ml/min; wavelength monitored, 210 nm. The compound eluted as two peaks with retention times of 0.9 min (salicylic acid), and 3.9 min (physostigmine).²

IR (KBr): 3320(broad), 2964, 2325, 1744, 1629, 1594, 1485, 1460, 1383, 1326, 1245, 1203, 1184, 1151, 1140, 1087, 1006, 993, 944, 860, 807, 754, 704, 667, 382 cm⁻¹.³ The IR spectrum was identical to that provided by the sponsors¹.

Source: Bill Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Washington, DC
Requested by LTC Jurgen von Bredow, PhD, MSC

¹Masamori E, Benitez A, and Lim P. Assay of physostigmine salicylate, WR-6570AM, BL25591. Menlo Park, CA: SRI International, 4 November 1986; Report no. 553.

²Wheeler CR. Toxicity testing of antidotes of chemical warfare agents. Laboratory notebook #85-12-024.1, pp 2-11. Letterman Army Institute of Research, Presidio of San Francisco, CA.

³Wheeler CR. Toxicity testing of antidotes of chemical warfare agents. Laboratory notebook #85-12-024.3, pp 10-11. Letterman Army Institute of Research, Presidio of San Francisco, CA.

APPENDIX A (cont.): Chemical Data

Analysis of Physostigmine Salicylate Dosing Solutions

The concentration of physostigmine salicylate in dosing solutions was determined by UV absorbance at 298 nm. Each solution was diluted and the absorbance measured by a Hitachi 110A Spectrophotometer. Using an extinction coefficient of 6222 L/moles·cm the concentration of physostigmine salicylate was calculated. The concentrations of dosing solutions for GLP study number 87008 are presented below:

| Date Sample Prepared | Date Sample Analyzed | Concentration Target | (mg/ml) Actual | % Target Conc. |
|----------------------|-------------------------|----------------------|----------------|----------------|
| 20 July 87 | 20 July 87* | 0.562 | 0.546 | 97.1 |
| 20 July 87 | 20 July 87 | 0.631 | 0.627 | 99.4 |
| 20 July 87 | 22 July 87 ⁺ | 0.631 | 0.611 | 96.8 |
| 22 July 87 | 22 July 87 | 0.665 | 0.654 | 98.3 |
| 22 July 87 | 22 July 87 | 0.708 | 0.694 | 98.0 |
| 22 July 87 | 22 July 87 | 0.750 | 0.737 | 98.3 |
| 8 Sep 87 | 8 Sep 87 [†] | 0.750 | 0.747 | 99.6 |
| 8 Sep 87 | 8 Sep 87 | 0.665 | 0.654 | 98.3 |
| 8 Sep 87 | 8 Sep 87 | 0.562 | 0.554 | 98.6 |
| 15 Sep 87 | 15 Sep 87 [§] | 0.891 | 0.863 | 96.9 |

*Wheeler CR. Toxicity testing of antidotes for chemical warfare agents. Laboratory Notebook #85-12-024, p 67. Letterman Army Institute of Research, Presidio of San Francisco, CA.

⁺Wheeler CR. Toxicity testing of antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.3, p 20. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[†]Wheeler CR. Toxicity testing of antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.1, p 66. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[§]Ibid. p 67.

APPENDIX B: Animal Data

Species: *Cavia porcellus*

Strain: Hartley

Source: Charles River Laboratories, Inc.
Kingston, NY

Sex: Male and female

Date of birth: Male: 19 June 1987
Female: 19 June and 5 August 1987

Method of randomization: Weight bias, stratified animal
allocation (Beckman TOXSYS® Animal
Allocation Program, SOP OP-ISG-24)

Animals in each group: 8 male and 16 female animals in
Groups 1, 3, and 5 (except 15 females in
Group 5); 8 male and 8 female animals in
Groups 2 and 4; 8 females in Groups 7 and
8; 5 male and 5 female animals for the
vehicle control (Group 6).

Condition of animals at start of study: Normal

Body weight range at dosing: 210 - 384 g

Identification procedures: Ear Tag.

Pretest conditioning: Quarantine/acclimation
Phase I: 8 - 20 July 1987
Phase II: 28 August - 7 September 1987

Justification: The guinea pig has proven to be a
sensitive and reliable animal model for
lethal dose determinations.

APPENDIX C: Historical Listing of Study Events

| <u>Date</u> | <u>Event</u> |
|----------------------|--|
| 7 Jul 87 | Received 57 male and 56 female Hartley guinea pigs. Animals were sexed and individually caged (Phase I). |
| 8 Jul 87 | Four animals (2 male and 2 female) were submitted for necropsy quality control. All animals were checked for physical condition, weighed, and given ear tags. |
| 8-20 Jul 87 | Animals were observed daily. |
| 13 Jul 87 | Animals were weighed and randomized into dose groups. |
| 20 Jul 87 | Animals (Groups 1 and 2) were weighed and removed from quarantine. Food was removed by 1800 |
| 21 Jul 87 | Animals (Groups 1 and 2) were weighed and dosed at approximately 0900. Observations were conducted at 1, 2, and 4 hours after dosing. Food was re-introduced 2-4 hours after dosing. |
| 22 Jul 87 | Animals (Groups 3 - 6) were weighed and removed from quarantine. Food was removed by 1800 |
| 23 Jul 87 | Animals (Groups 3 - 6) were weighed and dosed at approximately 0900. Observations were conducted at 1, 2, and 4 hours after dosing. Food was re-introduced 2-4 hours after dosing. |
| 22 Jul - 4 Aug 87 | Animals were observed for clinical signs in AM and PM (Groups 1 and 2). |
| 24 Jul - 6 Aug 87 | Animals were observed for clinical signs in AM and PM (Groups 3 - 6). |
| 28 Jul 87 | Animals were weighed (Groups 1 and 2). |
| 30 Jul 87 | Animals were weighed (Groups 3 - 6). |
| 3 Aug 87 | Food was removed by 1800 (Groups 1 and 2). |

APPENDIX C (cont.): Historical Listing of Study Events

| <u>Date</u> | <u>Event</u> |
|----------------------|--|
| 4 Aug 87 | Animals (Groups 1 and 2) were weighed and observed for clinical signs. Animals were delivered to Necropsy Suite. |
| 5 Aug 87 | Food was removed by 1800 (Groups 3 - 6). |
| 6 Aug 87 | Animals (Groups 3 - 6) were weighed and observed for clinical signs. Animals were delivered to Necropsy Suite. |
| 27 Aug 87 | Phase II animals (females) arrived and were sexed, observed for illness, and individually caged. Forty-one females were assigned to the study. |
| 28 Aug 87 | Animals were tagged and weighed, and one female QC animal was submitted to necropsy. |
| 28 Aug - 7 Sep 87 | Animals were observed daily while under quarantine. |
| 31 Aug 87 | Animals were weighed and randomized into dose groups. |
| 4 Sep 87 | All animals were weighed and removed from quarantine. |
| 7 Sep 87 | Food was removed by 1800 (Groups 1, 3, and 5). |
| 8 Sep 87 | Animals (Groups 1, 3, and 5) were weighed and dosed at approximately 0900. Observations were conducted 1, 2, and 4 hours after dosing. |
| 9-21 Sep 87 | Animals (Groups 1, 3, and 5) were observed for clinical signs in AM and PM |
| 9 Sep 87 | Food was removed by 1800 (Group 7). |
| 10 Sep 87 | Animals (Group 7) were weighed and dosed at approximately 0900. Observations were conducted 1, 2, and 4 hours after dosing. |
| 11-23 Sep 87 | Animals (Group 7) were observed for clinical signs in AM and PM. |

APPENDIX C (cont.): Historical Listing of Study Events

| <u>Date</u> | <u>Event</u> |
|-------------|--|
| 14 Sep 87 | Food (Group 8) was removed by 1800. |
| 15 Sep 87 | Animals (Groups 1, 3, and 5) were weighed. |
| 15 Sep 87 | Animals (Group 8) were weighed and dosed at approximately 0900. Observations were conducted 1, 2, and 4 hours after dosing. All died and were submitted to the Necropsy Suite on 15 Sep 87, except for # 240 and 248 which were sent to necropsy on 16 Sep 87. |
| 17 Sep 87 | Animals (Group 7) were weighed. |
| 21 Sep 87 | Food (Groups 1, 3, and 5) was removed by 1800. |
| 22 Sep 87 | Animals (Groups 1, 3, and 5) were weighed and observed for clinical signs at approximately 0730. Animals were delivered to Necropsy Suite. |
| 23 Sep 87 | Food (Group 7) was removed by 1800. |
| 24 Sep 87 | Animals (Group 7) were weighed and observed for clinical signs at approximately 0730. Animals were delivered to Necropsy Suite. |

APPENDIX D: Cumulative Mortality Data (Death/Group)
(8 Animals Per Group)

| Dose mg/kg | Hours | | | | Days | | | | | | | |
|----------------|----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | 1 | 2 | 4 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8-14 |
| Males | | | | | | | | | | | | |
| 5.62 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 6.31 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6.65 | 0 | 0 | 1 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 7.08 | 0 | 0 | 2 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 7.50 | 0 | 0 | 0 | 3 | 3 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 0* | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Females | | | | | | | | | | | | |
| 4.47 | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 5.62† | 1 | 1 | 3 | 7 | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 6.31 | 1 | 1 | 2 | 2 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 |
| 6.65† | 2 | 2 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 7.08 | 0 | 0 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 7.50§ | 0 | 0 | 4 | 7 | 8 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 8.91 | 0 | 0 | 4 | 8 | 3 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 0* | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 4 | 4 | 23 | 51 | 56 | 62 | 62 | 63 | 63 | 63 | 63 | 63 |

* 5 animals per group

† 16 animals per group

§ 15 animals per group

APPENDIX E: INDIVIDUAL ANIMAL HISTORIES

MALE: 5.62 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|--|---------------------------|------------------|
| 87E00116 | Stain, Brown, Perianal Increased Salivation | July 21 July 21 | Slight Slight |
| 87E00124 | Increased Salivation Death | July 21 July 23 | Slight 7.3 h |
| 87E00128 | Normal | N/A | N/A |
| 87E00133 | Stain, Yellow, Perianal | August 1 | Slight |
| 87E00140 | Death | July 21 | 4 h |
| 87E00147 | Stain, Brown, Perianal | July 21-31 August 2, 3 | Moderate |
| 87E00151 | Death | July 22 | 30 h |
| 87E00152 | Diarrhea | August 2, 3 | Present |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 6.31 mg/kg Thyroestigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|---|---|--|
| 87E00115 | Increased Salivation Death | July 21 July 23 | Present 43.2 h |
| 87E00132 | Increased Salivation | July 21 | Present |
| 87E00137 | Normal | N/A | N/A |
| 87E00146 | Tarry Feces | July 27,28 | Present |
| 87E00156 | Stain, Brown, Perianal Inactive Rough Coat Diarrhea Emaciated | July 21-30 July 25-30 July 26-Aug 3 July 29-Aug 3 Aug 1-3 | Slight Slight Present Present Moderate |
| 87E00163 | Normal | N/A | N/A |
| 87E00167 | Normal | N/A | N/A |
| 87E00170 | Stain, Brown, Perianal | July 21-25 | Slight |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 6.65 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|---------------------------------|-----------------------|-------------------|
| 87E00118 | Normal | N/A | N/A |
| 87E00120 | Death | July 23 | 6.6 h |
| 87E00136 | Normal | N/A | N/A |
| 87E00143 | Normal | N/A | N/A |
| 87E00144 | Death | July 24 | 24 h |
| 87E00149 | Stain, Brown, Perianal Death | July 23 July 23 | Moderate 4.0 h |
| 87E00168 | Increased Salivation | July 23 | Present |
| 87E00171 | Increased Salivation Death | July 23 July 23 | Present 5.5 h |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 7.08 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|---|--------------------------|------------------|
| 87E00122 | Death | July 23 | 3.6 h |
| 87E00123 | Death | July 23 | 3.6 h |
| 87E00127 | Stain, Brown, Perianal Stain, Yellow, Perianal | July 24 July 25-30 | Slight Slight |
| 87E00131 | Death | July 23 | 6.6 h |
| 87E00138 | Diarrhea | August 2,3 | Present |
| 87E00142 | Normal | N/A | N/A |
| 87E00159 | Death | July 24 | 24 h |
| 87E00165 | Stain, Brown, Perianal Stain, Yellow, Perianal | July 23 July 24-Aug 5 | Slight Slight |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 7.50 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|--|--|--|
| 87E00117 | Normal | N/A | N/A |
| 87E00119 | Normal | N/A | N/A |
| 87E00141 | Increased Salivation Death | July 23 July 23 | Present 6.2 h |
| 87E00150 | Increased Salivation Inactive Death | July 23 July 23 July 23 | Present Slight 4.7 h |
| 87E00153 | Tremors Inactive Rough Coat Death | July 24 July 24 July 24 July 25 | Moderate Moderate Moderate 48 h |
| 87E00154 | Inactive Diarrhea | July 23 August 5 | Slight Present |
| 87E00157 | Death | July 25 | 48 h |
| 87E00166 | Stain, Yellow, Perianal Death | July 23 July 23 | Slight 5.6 h |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: Vehicle Control

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|------------------|------------------------------------|--------------------------|-------------------|
| 87E00121 | Normal | N/A | N/A |
| 87E00125 | Normal | N/A | N/A |
| 87E00135 | Stain, Brown, Perianal Diarrhea | July 23 Aug 4, 5 | Slight Present |
| 87E00155 | Normal | N/A | N/A |
| 87E00158 | Normal | N/A | N/A |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 5.62 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|--|-------------------------------------|-------------------------------|
| 87E00172 | Stain, Brown, Perianal Increased Salivation Diarrhea | July 21-31 July 21 July 27-31 | Slight Moderate Present |
| 87E00177 | Normal | N/A | N/A |
| 87E00186 | Death | July 21 | 0.1 h |
| 87E00201 | Normal | N/A | N/A |
| 87E00211 | Stain, Brown, Perianal Increased Salivation Death | July 21 July 21 July 23 | Slight Slight 47 h |
| 87E00219 | Normal | N/A | N/A |
| 87E00226 | Normal | N/A | N/A |
| 87E00227 | Stain, Brown, Perianal Death | July 21 July 21 | Slight 4.5 h |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 5.62 mg/kg Physostigmine salicylate (cont.)

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|-------------------------------|-----------------------|-----------------|
| 87E00234 | Increased Salivation | Sep 8 | Slight |
| 87E00237 | Increased Salivation Death | Sep 8 Sep 8 | Slight 7.1 h |
| 87E00242 | Increased Salivation Death | Sep 8 Sep 8 | Slight 6.4 h |
| 87E00249 | Stain, Yellow, Perianal | Sep 19-21 | Slight |
| 87E00255 | Death | Sep 8 | 3.7 h |
| 87E00256 | Diarrhea Death | Sep 8 Sep 8 | Slight 3.6 h |
| 87E00261 | Diarrhea | Sep 9, 15-21 | Moderate |
| | Prolapsed Uterus | Sep 10 | Moderate |
| | Uterus, Necrosis | Sep 10 | Slight |
| | Uterus, Sutured | Sep 10 | Present |
| | Drainage, Brown, Vulva | Sep 12, 13 | Slight |
| | Drainage, Yellow, Vulva | Sep 17 | Moderate |
| | Material, Brown, Abdomen | Sep 19, 20 | Moderate |
| | Material, Brown, Hind Legs | Sep 19, 20 | Moderate |
| | Rough Coat | Sep 19-21 | Moderate |
| 87E00266 | Death | Sep 8 | 6.2 h |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 6.31 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|--|---|---|
| 87E00188 | Stain, Brown, Perianal Death | July 21 July 21 | Slight 4.0 h |
| 87E00193 | Stain, Brown, Perianal Increased Salivation Death | July 21 July 21 July 22 | Slight Present 18 h |
| 87E00195 | Normal | N/A | N/A |
| 87E00202 | Death | July 21 | 0.1 h |
| 87E00203 | Death | July 22 | 24 h |
| 87E00222 | Stain, Brown, Perianal Diarrhea | July 21-Aug 3 July 29 | Moderate Present |
| 87E00224 | Stain, Brown, Perianal | July 21 | Slight |
| 87E00228 | Increased Salivation Inactive Emaciated Rough Coat Death | July 21 July 23, 24 July 24 July 24 July 25 | Present Moderate Present Present 4 days |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 6.65 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|--|-------------------------------|----------------------------|
| 87E00180 | Tremors Increased Salivation Death | July 23 July 23 July 23 | Slight Present 5.4 h |
| 87E00183 | Death | July 23 | 3.8 h |
| 87E00184 | Not Dosed | N/A | N/A |
| 87E00185 | Increased Salivation Death | July 23 July 23 | Present 5.4 h |
| 87E00189 | Death | July 23 | 0.03 h |
| 87E00191 | Death | July 23 | 0.02 h |
| 87E00197 | Death | July 23 | 6.3 h |
| 87E00213 | Normal | N/A | N/A |
| 87E00221 | Death | July 23 | 2.9 h |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 6.65 mg/kg Physostigmine salicylate (cont.)

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|---|-------------------------|-----------------------------|
| 87E00231 | Increased Salivation | Sep 8 | Slight |
| 87E00233 | Death | Sep 8 | 2.6 h |
| 87E00241 | Hunched Posture Stain, Yellow, Abdomen | Sep 8 Sep 9-21 | Present Moderate |
| 87E00251 | Increased Salivation Rough Coat | Sep 8 Sep 9 | Slight Slight |
| 87E00262 | Normal | N/A | N/A |
| 87E00265 | Ataxia Increased Salivation Death | Sep 8 Sep 8 Sep 8 | Marked Moderate 2.4 h |
| 87E00267 | Increased Salivation | Sep 8 | Slight |
| 87E00269 | Increased Salivation Rough Coat | Sep 8 Sep 9 | Slight Slight |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 7.08 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|-------------------------------|-----------------------|------------------|
| 87E00173 | Increased Salivation | July 23 | Present |
| 87E00174 | Increased Salivation Death | July 23 July 23 | Present 5.0 h |
| 87E00175 | Death | July 23 | 6.4 h |
| 87E00178 | Increased Salivation Death | July 23 July 23 | Present 4.9 h |
| 87E00194 | Death | July 23 | 4.9 h |
| 87E00206 | Normal | N/A | N/A |
| 87E00218 | Increased Salivation Death | July 23 July 23 | Slight 4.8 h |
| 87E00220 | Increased Salivation | July 23 | Slight |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 7.50 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|---|--------------------------------------|-------------------------------|
| 87E00198 | Normal | N/A | N/A |
| 87E00199 | Tremors Death | July 23 July 23 | Slight 4.6 h |
| 87E00204 | Stain, Yellow, Perianal Increased Salivation Stain, Brown, Perianal | July 23, Aug 5 July 23 Aug 1,2 | Slight Present Moderate |
| 87E00205 | Stain, Yellow, Perianal Death | July 23 July 23 | Slight 4.6 h |
| 87E00207 | Death | July 24 | 24 h |
| 87E00212 | Stain, Brown, Perianal | July 23 | Slight |
| 87E00216 | Normal | N/A | N/A |
| 87E00221 | To Group 3 | N/A | N/A |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 7.50 mg/kg Physostigmine salicylate (cont.)

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|------------------------|-----------------------|----------|
| 87E00229 | Inactive | Sep 8 | Moderate |
| | Ataxia | Sep 8 | Moderate |
| | Death | Sep 8 | 2.4 h |
| 87E00239 | Paralysis, Hind Limb | Sep 9 | Marked |
| | Moribund | Sep 9 | Present |
| | Death | Sep 10 | 48 h |
| 87E00244 | Increased Salivation | Sep 8 | Moderate |
| 87E00247 | Increased Salivation | Sep 8 | Slight |
| | Ataxia | Sep 8 | Slight |
| | Death | Sep 8 | 2.5 h |
| 87E00250 | Increased Salivation | Sep 8 | Moderate |
| | Lacrimation | Sep 8 | Slight |
| | Death | Sep 8 | 4.6 h |
| 87E00258 | Increased Salivation | Sep 8 | Moderate |
| | Ataxia | Sep 8 | Slight |
| | Death | Sep 8 | 2.4 h |
| 87E00259 | Increased Salivation | Sep 8 | Slight |
| | Ataxia | Sep 8 | Moderate |
| | Tremors | Sep 8 | Slight |
| | Death | Sep 8 | 2.3 h |
| 87E00263 | Increased Salivation | Sep 8 | Slight |
| | Stain, Yellow, Abdomen | Sep 16-21 | Slight |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Vehicle Controls

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|---------------------------------|--------------------------------|-------------------|
| 87E00176 | Stain, Brown, Perianal Diarrhea | July 23 July 29, Aug 1-3 | Slight Present |
| 87E00182 | Normal | N/A | N/A |
| 87E00200 | Stain, Brown, Perianal Diarrhea | July 23 Aug 2, 3 | Slight Present |
| 87E00215 | Normal | N/A | N/A |
| 87E00225 | Normal | N/A | N/A |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4.47 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|---|--|---|
| 87E00230 | Increased Salivation | Sep 10 | Slight |
| 87E00236 | Increased Salivation Death | Sep 10 Sep 10 | Moderate 6.3 h |
| 87E00245 | Increased Salivation Stain, Brown, Perianal Stain, Brown, Abdomen Stain, Yellow, Abdomen | Sep 10 Sep 12, 13 Sep 16, 17 Sep 19-21, 23 | Moderate Moderate Slight Slight |
| 87E00252 | Increased Salivation Stain, Brown, Perianal | Sep 10 Sep 16-23 | Slight Marked |
| 87E00253 | Increased Salivation Pallor Inactive Material, Brown, Fr. Leg Diarrhea | Sep 10 Sep 12-16 Sep 14-16 Sep 20-23 Sep 21-23 | Slight Moderate Moderate Moderate Present |
| 87E00257 | Increased Salivation Loss of Equilibrium Inactive Pale Skin Color Death | Sep 10 Sep 11 Sep 11 Sep 11 Sep 12 | Slight Moderate Moderate Present 48 h |
| 87E00260 | Normal | N/A | N/A |
| 87E00264 | Increased Salivation Stain, Brown, Abdomen Stain, Yellow, Abdomen | Sep 10 Sep 12-17 Sep 19-23 | Slight Moderate Slight |

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 8.91 mg/kg Physostigmine salicylate

| Animal Number | Clinical Signs | Dates Observed (1987) | Severity |
|---------------|---|--------------------------------------|---|
| 87E00235 | Increased Salivation Death | Sep 15 Sep 15 | Slight 4.0 h |
| 87E00238 | Death | Sep 15 | 6.9 h |
| 87E00240 | Increased Salivation Death | Sep 15 Sep 15 | Slight 7.1 h |
| 87E00243 | Increased Salivation Death | Sep 15 Sep 15 | Moderate 3.8 h |
| 87E00246 | Increased Salivation Death | Sep 15 Sep 15 | Slight 6.7 h |
| 87E00248 | Increased Salivation Death | Sep 15 Sep 15 | Slight 6.7 h |
| 87E00254 | Increased Salivation Ataxia Inactive Death | Sep 15 Sep 15 Sep 15 Sep 15 | Moderate Moderate Moderate 2.6 h |
| 87E00268 | Death | Sep 15 | 3.7 h |

APPENDIX F: Individual Body Weights

MALES 5.62 mg/kg - Group 1

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|--------------------|------------|-----------|-------|---------------------|
| 87E00116 | 230† | 300 | 381 | 384 |
| 87E00124 | 258 | 335 | - | - |
| 87E00128 | 244 | 330 | 377 | 389 |
| 87E00133 | 237 | 297 | 375 | 387 |
| 97E00140 | 222 | 266 | - | - |
| 87E00147 | 239 | 308 | 257 | 284 |
| 87E00151 | 216 | 293 | - | - |
| 87E00152 | 235 | 295 | 358 | 359 |
| Mean | 235.1 | 303.0 | 349.6 | 360.6 |
| Standard Deviation | 13.0 | 21.9 | 52.5 | 44.5 |
| Std. Error of Mean | 4.6 | 7.7 | 23.5 | 19.9 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 6.31 mg/kg - Group 2

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|------------------|--------------|-------|------------------------|
| 87E00115 | 235 [†] | 289 | - | - |
| 87E00132 | 223 | 292 | 357 | 371 |
| 87E00137 | 236 | 310 | 375 | 387 |
| 87E00146 | 199 | 303 | 375 | 391 |
| 87E00156 | 235 | 291 | 233 | 206 |
| 87E00163 | 216 | 279 | 339 | 361 |
| 87E00167 | 258 | 322 | 295 | 325 |
| 87E00170 | 239 | 315 | 376 | 388 |
| Mean | 230.1 | 300.1 | 335.7 | 347.0 |
| Standard Deviation | 17.6 | 14.8 | 53.8 | 66.3 |
| Std. Error of Mean | 6.2 | 5.2 | 20.3 | 25.1 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 6.65 mg/kg - Group 3

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|---------------|--------------|-------|------------------------|
| 87E00118 | 238† | 305 | 341 | 351 |
| 87E00120 | 252 | 324 | - | - |
| 87E00136 | 233 | 306 | 351 | 362 |
| 87E00143 | 233 | 351 | 419 | 431 |
| 87E00144 | 196 | 303 | - | - |
| 87E00149 | 195 | 210 | - | - |
| 87E00168 | 240 | 329 | 391 | 420 |
| 87E00171 | 238 | 308 | - | - |
| Mean | 228.1 | 304.5 | 375.5 | 391.0 |
| Standard Deviation | 21.0 | 41.6 | 36.2 | 40.3 |
| Std. Error of Mean | 7.4 | 14.7 | 18.1 | 20.2 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 7.08 mg/kg - Group 4

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|------------------|--------------|-------|------------------------|
| 87E00122 | 220 [†] | 298 | - | - |
| 87E00123 | 252 | 339 | - | - |
| 87E00127 | 230 | 298 | 351 | 368 |
| 87E00131 | 236 | 321 | - | - |
| 87E00138 | 218 | 304 | 367 | 389 |
| 87E00142 | 202 | 289 | 341 | 351 |
| 87E00159 | 247 | 310 | - | - |
| 87E00165 | 226 | 273 | 338 | 346 |
| Mean | 228.9 | 304.0 | 349.3 | 363.5 |
| Standard Deviation | 16.2 | 20.0 | 13.0 | 19.4 |
| Std. Error of Mean | 5.7 | 7.1 | 6.5 | 9.7 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 7.50 mg/kg - Group 5

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|---------------|--------------|-------|------------------------|
| 87E00117 | 236† | 302 | 355 | 374 |
| 87E00119 | 248 | 327 | 398 | 410 |
| 87E00141 | 252 | 306 | - | - |
| 87E00150 | 214 | 330 | - | - |
| 87E00153 | 226 | 313 | - | - |
| 87E00154 | 218 | 269 | 325 | 315 |
| 87E00157 | 200 | 265 | - | - |
| 87E00166 | 237 | 301 | - | - |
| Mean | 228.9 | 301.4 | 359.3 | 366.3 |
| Standard Deviation | 17.7 | 24.3 | 36.7 | 48.0 |
| Std. Error of Mean | 6.3 | 8.6 | 21.2 | 27.7 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 5.62 mg/kg - Group 1

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|---------------|--------------|-------|------------------------|
| 87E00172 | 244† | 279 | 333 | 338 |
| 87E00177 | 233 | 262 | 317 | 322 |
| 87E00186 | 216 | 280 | - | - |
| 87E00201 | 209 | 263 | 324 | 303 |
| 87E00211 | 248 | 386 | - | - |
| 87E00219 | 238 | 299 | 352 | 370 |
| 87E00226 | 226 | 266 | 314 | 309 |
| 87E00227 | 229 | 282 | - | - |
| 87E00234 | 211 | 308 | 369 | 372 |
| 87E00237 | 259 | 312 | - | - |
| 87E00242 | 254 | 276 | - | - |
| 87E00249 | 209 | 278 | 360 | 357 |
| 87E00255 | 237 | 294 | - | - |
| 87E00256 | 233 | 263 | - | - |
| 87E00261 | 211 | 238 | 229 | 246 |
| 87E00266 | 273 | 314 | - | - |
| Mean | 233.2 | 287.5 | 324.8 | 327.1 |
| Standard Deviation | 19.3 | 33.4 | 43.7 | 42.0 |
| Std. Error of Mean | 4.8 | 8.4 | 15.4 | 14.9 |

*Weights after an overnight fast.

†Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 6.31 mg/kg - Group 2

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|--------------------|------------|-----------|-------|---------------------|
| 87E00188 | 222† | 252 | - | - |
| 87E00193 | 217 | 263 | - | - |
| 87E00195 | 231 | 273 | 323 | 337 |
| 87E00202 | 265 | 326 | - | - |
| 87E00203 | 220 | 255 | - | - |
| 87E00222 | 229 | 260 | 314 | 315 |
| 87E00224 | 239 | 295 | 347 | 351 |
| 87E00228 | 218 | 263 | - | - |
| Mean | 230.1 | 273.4 | 328.0 | 334.3 |
| Standard Deviation | 16.0 | 25.1 | 17.1 | 18.1 |
| Std. Error of Mean | 5.6 | 8.9 | 9.8 | 10.5 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 6.65 mg/kg - Group 3

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|---------------|--------------|-------|------------------------|
| 87E00180 | 226† | 283 | - | - |
| 87E00183 | 233 | 299 | - | - |
| 87E00185 | 205 | 272 | - | - |
| 87E00189 | 225 | 304 | - | - |
| 87E00191 | 235 | 301 | - | - |
| 87E00197 | 258 | 309 | - | - |
| 87E00213 | 235 | 289 | 331 | 338 |
| 87E00221 | 191 | 277 | - | - |
| 87E00231 | 237 | 294 | 358 | 377 |
| 87E00233 | 198 | 284 | - | - |
| 87E00241 | 296 | 324 | 384 | 385 |
| 87E00251 | 273 | 300 | 341 | 358 |
| 87E00262 | 249 | 266 | 336 | 339 |
| 87E00265 | 222 | 298 | - | - |
| 87E00267 | 246 | 284 | 341 | 346 |
| 87E00269 | 233 | 252 | 295 | 301 |
| Mean | 235.1 | 289.8 | 340.9 | 349.1 |
| Standard Deviation | 26.6 | 17.8 | 27.0 | 28.0 |
| Std. Error of Mean | 6.6 | 4.4 | 10.2 | 10.6 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 7.03 mg/kg - Group 4

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|--------------------|------------|-----------|-------|---------------------|
| 87E00173 | 237† | 289 | 336 | 336 |
| 87E00174 | 243 | 288 | - | - |
| 87E00175 | 232 | 305 | - | - |
| 87E00178 | 253 | 299 | - | - |
| 87E00194 | 237 | 272 | - | - |
| 87E00206 | 235 | 280 | 352 | 351 |
| 87E00218 | 238 | 291 | - | - |
| 87E00220 | 215 | 277 | 334 | 314 |
| Mean | 236.3 | 287.6 | 340.7 | 333.7 |
| Standard Deviation | 10.7 | 11.1 | 9.9 | 18.6 |
| Std. Error of Mean | 3.8 | 3.9 | 5.7 | 10.7 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 7.50 mg/kg - Group 5

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|---------------|--------------|-------|------------------------|
| 87E00198 | 237† | 271 | 345 | 340 |
| 87E00199 | 241 | 289 | - | - |
| 87E00204 | 225 | 244 | 274 | 286 |
| 87E00205 | 231 | 281 | - | - |
| 87E00207 | 241 | 306 | - | - |
| 87E00212 | 244 | 238 | 295 | 305 |
| 87E00216 | 236 | 309 | 383 | 390 |
| 87E00279 | 225 | 239 | - | - |
| 87E00239 | 254 | 313 | - | - |
| 87E00244 | 270 | 303 | 356 | 369 |
| 87E00247 | 277 | 294 | - | - |
| 87E00250 | 254 | 267 | - | - |
| 87E00258 | 217 | 281 | - | - |
| 87E00259 | 236 | 268 | - | - |
| 87E00263 | 255 | 268 | 324 | 321 |
| Mean | 242.9 | 278.1 | 329.5 | 335.2 |
| Standard Deviation | 16.6 | 24.8 | 40.3 | 39.2 |
| Std. Error of Mean | 4.3 | 6.4 | 16.4 | 16.0 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights**VEHICLE CONTROLS - Group 6**

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|------------------|--------------|-------|------------------------|
| MALES | | | | |
| 87E00121 | 223 [†] | 291 | 361 | 349 |
| 87E00125 | 238 | 326 | 386 | 403 |
| 87E00135 | 233 | 326 | 406 | 431 |
| 87E00155 | 232 | 299 | 359 | 369 |
| 87E00158 | 221 | 283 | 346 | 349 |
| Mean | 229.4 | 305.0 | 371.6 | 380.2 |
| Standard Deviation | 7.2 | 20.0 | 24.1 | 36.0 |
| Std. Error of Mean | 3.7 | 8.9 | 10.8 | 16.1 |
| FEMALES | | | | |
| 87E00176 | 225 | 278 | 328 | 326 |
| 87E00182 | 188 | 265 | 320 | 322 |
| 87E00200 | 222 | 299 | 336 | 325 |
| 87E00215 | 226 | 275 | 324 | 330 |
| 87E00225 | 223 | 267 | 339 | 343 |
| Mean | 216.8 | 276.8 | 329.4 | 329.2 |
| Standard Deviation | 16.2 | 13.5 | 8.0 | 8.2 |
| Std. Error of Mean | 7.2 | 6.0 | 3.6 | 3.7 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 4.47 mg/kg - Group 7

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|---------------|--------------|-------|------------------------|
| 87E00230 | 208† | 277 | 322 | 333 |
| 87E00236 | 277 | 384 | - | - |
| 87E00245 | 241 | 294 | 349 | 359 |
| 87E00252 | 250 | 292 | 356 | 360 |
| 87E00253 | 226 | 273 | 225 | 207 |
| 87E00257 | 208 | 290 | - | - |
| 87E00260 | 215 | 244 | 302 | 257 |
| 87E00264 | 255 | 296 | 352 | 364 |
| Mean | 235.0 | 293.8 | 317.7 | 313.3 |
| Standard Deviation | 25.0 | 40.3 | 50.0 | 65.9 |
| Std. Error of Mean | 8.8 | 14.2 | 20.4 | 26.9 |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 8.91 mg/kg - Group 8

| Animal No. | At Receipt | At Dosing | Day 7 | Termination Day 14* |
|-----------------------|---------------|--------------|-------|------------------------|
| 87E00235 | 283† | 378 | - | - |
| 87E00238 | 225 | 348 | - | - |
| 87E00240 | 290 | 359 | - | - |
| 87E00243 | 252 | 334 | - | - |
| 87E00246 | 232 | 368 | - | - |
| 87E00248 | 242 | 299 | - | - |
| 87E00254 | 230 | 317 | - | - |
| 87E00268 | 268 | 336 | - | - |
| Mean | 252.8 | 342.4 | - | - |
| Standard Deviation | 25.0 | 26.4 | - | - |
| Std. Error of Mean | 8.8 | 9.3 | - | - |

* Weights after an overnight fast.

† Weights are given in grams.

APPENDIX G: Pathology Report

Pathology Report GLP 87008

Acute Oral Toxicity

I. Compound: Physostigmine salicylate
Species: Cavia porcellus, Hartley, young adult.

II. Principal Investigator: CPT Denzil F. Frost
Pathologist: MAJ Charles B. Clifford

III. Comment: No gross lesions were observed in animals surviving the fourteen days of the study. Gross observations in cases of unscheduled deaths are compatible with either generalized increased parasympathetic tone (perioral staining and intussusception) or common incidental findings (hepatic necrosis) of little clinical significance in guinea pigs. No evidence of direct tissue damage due to physostigmine salicylate was observed in any animal.

Charles B. Clifford

CHARLES B. CLIFFORD, DVM
MAJ, VC
Division of Pathology

12 November 1987/dbj

APPENDIX G (cont.): Pathology Report**ATTACHMENT:**

GROUP #1 (5.62 mg/kg) 16 animals, dosed 21 Jul 87, 10 animals sacrificed 4 Aug 87.

| <u>ANIMAL ID#</u> | <u>LAIR ACC#</u> | <u>SEX</u> | <u>NECROPSY DATE</u> | <u>DIAGNOSES</u> |
|-------------------|------------------|------------|----------------------|-----------------------|
| 87E00116 | 41444 | M | 4 Aug 87 | No lesions recognized |
| 128 | 41445 | M | 4 Aug 87 | No lesions recognized |
| 133 | 41447 | M | 4 Aug 87 | No lesions recognized |
| 147 | 41450 | M | 4 Aug 87 | No lesions recognized |
| 152 | 41451 | M | 4 Aug 87 | No lesions recognized |
| 172 | 41456 | F | 4 Aug 87 | No lesions recognized |
| 177 | 41457 | F | 4 Aug 87 | No lesions recognized |
| 201 | 41459 | F | 4 Aug 87 | No lesions recognized |
| 219 | 41460 | F | 4 Aug 87 | No lesions recognized |
| 226 | 41463 | F | 4 Aug 87 | No lesions recognized |

GROUP #1 Spontaneous deaths, 6 animals

| | | | | |
|----------|-------|---|-----------|-------------------------|
| 87E00124 | 41344 | M | 21 Jul 87 | Serous oral discharge |
| 140 | 41343 | M | 21 Jul 87 | Serous oral discharge |
| 151 | 41348 | M | 22 Jul 87 | Brown perioral staining |
| 186 | 41341 | F | 21 Jul 87 | No lesions recognized |
| 211 | 42065 | F | 23 Jul 87 | No lesions recognized |
| 227 | 41342 | F | 21 Jul 87 | Serous oral discharge |

GROUP #2 (6.31 mg/kg), 16 animals, dosed 21 Jul 87, 10 animals sacrificed 4 Aug 87.

| | | | | |
|----------|-------|---|----------|-----------------------|
| 87E00132 | 41446 | M | 4 Aug 87 | No lesions recognized |
| 137 | 41448 | M | 4 Aug 87 | No lesions recognized |
| 146 | 41449 | M | 4 Aug 87 | No lesions recognized |
| 156 | 41452 | M | 4 Aug 87 | No lesions recognized |
| 163 | 41453 | M | 4 Aug 87 | No lesions recognized |
| 167 | 41454 | M | 4 Aug 87 | No lesions recognized |
| 170 | 41455 | M | 4 Aug 87 | No lesions recognized |
| 195 | 41458 | F | 4 Aug 87 | No lesions recognized |
| 222 | 41461 | F | 4 Aug 87 | No lesions recognized |
| 224 | 41462 | F | 4 Aug 87 | No lesions recognized |

GROUP #2 Spontaneous deaths, 6 animals

| | | | | |
|----------|-------|---|-----------|---|
| 87E00115 | 41349 | M | 23 Jul 87 | No lesions recognized |
| 188 | 41347 | F | 22 Jul 87 | No lesions recognized |
| 193 | 41345 | F | 22 Jul 87 | Serous oral discharge, mild, focal, hepatic necrosis. |
| 202 | 41340 | F | 21 Jul 87 | Ingesta present around and in mouth, mild hemopericardium, mild, focal, hepatic necrosis. |
| 203 | 41346 | F | 22 Jul 87 | No lesions recognized |
| 228 | 41378 | F | 25 Jul 87 | No lesions recognized |

APPENDIX G (cont.): Pathology Report

GROUP #3 (6.65 mg/kg), 24 animals, dosed 23 Jul 87, 5 animals sacrificed 6 Aug 87.

| <u>ANIMAL ID#</u> | <u>LAIR ACC#</u> | <u>SEX</u> | <u>NECROPSY DATE</u> | <u>DIAGNOSES</u> |
|-------------------|------------------|------------|----------------------|-----------------------|
| 87E00118 | 41482 | M | 6 Aug 87 | No lesions recognized |
| 136 | 41488 | M | 6 Aug 87 | No lesions recognized |
| 143 | 41491 | M | 6 Aug 87 | No lesions recognized |
| 168 | 41496 | M | 6 Aug 87 | No lesions recognized |
| 213 | 41505 | F | 6 Aug 87 | No lesions recognized |

GROUP #3 Spontaneous deaths, 13 animals, #1

| | | | | |
|----------|-------|---|-----------|---|
| 87E00120 | 41371 | M | 23 Jul 87 | Serous oral discharge |
| 144 | 41375 | M | 24 Jul 87 | No lesions recognized |
| 149 | 41356 | M | 23 Jul 87 | Yellow-brown perioral and perianal staining |
| 171 | 41368 | M | 23 Jul 87 | No lesions recognized |
| 180 | 41360 | F | 23 Jul 87 | Serous oral discharge |
| 183 | 41354 | F | 23 Jul 87 | Serous oral discharge |
| 185 | 41361 | F | 23 Jul 87 | Serous oral discharge |
| 189 | 42064 | F | 23 Jul 87 | No lesions recognized |
| 191 | 42063 | F | 23 Jul 87 | Brown perinasal staining |
| 197 | 41369 | F | 23 Jul 87 | Ingesta around oral cavity |
| 221 | 41357 | F | 23 Jul 87 | Ingesta around oral cavity |

#1 Additionally, 87F00184 (Accession #41464), a female was not dosed due to excessive weight loss, and was found dead 30 Jul 87. Gross observations included extensive matting of fur with diarrhea, severe dehydration and emaciation.

GROUP #4 (7.08 mg/kg), 9 animals dosed 23 Jul 87. Seven animals sacrificed 6 Aug 87.

| | | | | |
|----------|-------|---|----------|-----------------------|
| 87E00127 | 41486 | M | 6 Aug 87 | No lesions recognized |
| 138 | 41489 | M | 6 Aug 87 | No lesions recognized |
| 142 | 41490 | M | 6 Aug 87 | No lesions recognized |
| 165 | 41495 | M | 6 Aug 87 | No lesions recognized |
| 173 | 41497 | F | 6 Aug 87 | No lesions recognized |
| 206 | 41503 | F | 6 Aug 87 | No lesions recognized |
| 220 | 41508 | F | 6 Aug 87 | No lesions recognized |

APPENDIX G (cont.): Pathology Report

GROUP #4 Spontaneous deaths, 9 animals

| <u>ANIMAL ID#</u> | <u>LAIR ACC#</u> | <u>SEX</u> | <u>NECROPSY DATE</u> | <u>DIAGNOSES</u> |
|-------------------|------------------|------------|----------------------|--|
| 87E00122 | 41358 | M | 23 Jul 87 | Serous oral discharge |
| 123 | 41355 | M | 23 Jul 87 | Serous oral discharge |
| 131 | 41372 | M | 23 Jul 87 | Serous oral discharge |
| 159 | 41376 | M | 24 Jul 87 | Serous oral and ocular discharge |
| 174 | 41362 | F | 23 Jul 87 | Brown perioral staining |
| 175 | 41374 | F | 23 Jul 87 | Green-brown perioral and perianal staining |
| 178 | 41364 | F | 23 Jul 87 | Serous oral discharge |
| 194 | 41367 | F | 23 Jul 87 | Serous oral discharge |
| 218 | 41363 | F | 23 Jul 87 | Serous oral discharge |

GROUP #5 (7.5 mg/kg) 15 animals, dosed 23 Jul 87. 7 animals sacrificed 6 Aug 87.

| | | | | |
|----------|-------|---|----------|-----------------------|
| 87E00117 | 41481 | M | 6 Aug 87 | No lesions recognized |
| 119 | 41483 | M | 6 Aug 87 | No lesions recognized |
| 154 | 41492 | M | 6 Aug 87 | No lesions recognized |
| 198 | 41500 | F | 6 Aug 87 | No lesions recognized |
| 204 | 41502 | F | 6 Aug 87 | No lesions recognized |
| 212 | 41504 | F | 6 Aug 87 | No lesions recognized |
| 216 | 41507 | F | 6 Aug 87 | No lesions recognized |

GROUP #5 Spontaneous deaths, 8 animals

| | | | | |
|----------|-------|---|-----------|-----------------------------------|
| 87E00141 | 41373 | M | 23 Jul 87 | Serous oral discharge |
| 150 | 41366 | M | 23 Jul 87 | No lesions recognized |
| 153 | 41379 | M | 25 Jul 87 | Multifocal, mild hepatic necrosis |
| 157 | 41380 | M | 25 Jul 87 | No lesions recognized |
| 166 | 41370 | M | 23 Jul 87 | Serous oral discharge |
| 199 | 41365 | F | 23 Jul 87 | Serous oral discharge |
| 205 | 41359 | F | 23 Jul 87 | Serous oral discharge |
| 207 | 41377 | F | 24 Jul 87 | No lesions recognized |

GROUP #6 (Control Group 0 mg/kg) 10 animals, all sacrificed 6 Aug 87

| | | | | |
|----------|-------|---|----------|-----------------------|
| 87E00121 | 41484 | M | 6 Aug 87 | No lesions recognized |
| 125 | 41485 | M | 6 Aug 87 | No lesions recognized |
| 135 | 41487 | M | 6 Aug 87 | No lesions recognized |
| 155 | 41493 | M | 6 Aug 87 | No lesions recognized |
| 158 | 41494 | M | 6 Aug 87 | No lesions recognized |
| 176 | 41498 | F | 6 Aug 87 | No lesions recognized |
| 182 | 41499 | F | 6 Aug 87 | No lesions recognized |
| 200 | 41501 | F | 6 Aug 87 | No lesions recognized |
| 215 | 41506 | F | 6 Aug 87 | No lesions recognized |
| 225 | 41509 | F | 6 Aug 87 | No lesions recognized |

APPENDIX G (cont.): Pathology Report

Additional Animals:

GROUP #1 (5.62 mg/kg), 8 animals, dosed 8 Sep 87. Three animals sacrificed 22 Sep 87.

| <u>ANIMAL ID#</u> | <u>LAIR ID#</u> | <u>SEX</u> | <u>DIAGNOSES</u> |
|-------------------|-----------------|------------|-----------------------|
| 87E00234 | 41748 | F | No lesions recognized |
| 249 | 41749 | F | No lesions recognized |
| 261 | 41750 | F | Weight loss, diarrhea |

Spontaneous Deaths, 5 animals

| | | | |
|-----|-------|---|--|
| 237 | 41711 | F | No lesions recognized. Necropsied 9 Sept 1987. |
| 242 | 41710 | F | No lesions recognized. Necropsied 9 Sept 1987. |
| 255 | 41707 | F | No lesions recognized. Necropsied 8 Sept 1987. |
| 256 | 41708 | F | No lesions recognized. Necropsied 8 Sept 1987. |
| 266 | 41712 | F | No lesions recognized. Necropsied 9 Sept 1987. |

GROUP #3 (6.65 mg/kg), 8 animals, dosed 8 Sep 87. Six animals sacrificed 22 Sep 87.

| | | | |
|----------|-------|---|-----------------------|
| 87E00231 | 41742 | F | No lesions recognized |
| 241 | 41743 | F | No lesions recognized |
| 251 | 41744 | F | No lesions recognized |
| 262 | 41745 | F | No lesions recognized |
| 267 | 41746 | F | No lesions recognized |
| 269 | 41747 | F | No lesions recognized |

Spontaneous Deaths, 2 animals. Necropsied: 8 Sep 87

| | | | |
|-----|-------|---|-----------------------|
| 233 | 41701 | F | No lesions recognized |
| 265 | 41702 | F | No lesions recognized |

GROUP #5 (7.5 mg/kg), 8 animals, dosed 8 Sep 87. Three animals sacrificed 22 Sep 87.

| | | | |
|----------|-------|---|-----------------------|
| 87E00244 | 41740 | F | No lesions recognized |
| 250 | 41709 | F | No lesions recognized |
| 263 | 41741 | F | No lesions recognized |

Spontaneous Deaths, 5 animals. (Necropsied 8 Sept 87 unless otherwise stated.)

| | | | |
|-----|-------|---|--|
| 229 | 41705 | F | No lesions recognized. |
| 239 | 41713 | F | 10 cm intussusception at ileocolic junction, with necrosis and hemorrhage. Necropsied 10 Sep 87. |
| 247 | 41704 | F | No lesions recognized. |
| 258 | 41703 | F | No lesions recognized. |
| 259 | 41706 | F | No lesions recognized. |

APPENDIX G (cont.): Pathology Report

GROUP #7 (4.47 mg/kg), 8 animals, dosed 10 Sep 87. Six animals sacrificed 24 Sep 87.

| <u>ANIMAL ID#</u> | <u>LAIR ID#</u> | <u>SEX</u> | <u>DIAGNOSES</u> |
|-------------------|-----------------|------------|--------------------------------|
| 87E00230 | 41755 | F | No lesions recognized |
| 245 | 41756 | F | No lesions recognized |
| 252 | 41757 | F | No lesions recognized |
| 253 | 41758 | F | Diarrhea, diminished body fat. |
| 260 | 41759 | F | No lesions recognized |
| 264 | 41760 | F | No lesions recognized |

Spontaneous Deaths, 2 animals

| | | | |
|-----|-------|---|---|
| 236 | 41719 | F | No lesions recognized. Necropsied 11 Sept 1987. |
| 257 | 41720 | F | Ileocolic intussusception with necrosis of the intussusceptum. Necropsied 14 Sept 1987. |

GROUP #8 (8.91 mg/kg), 8 animals, dosed 15 Sep 87. All spontaneous deaths.

| | | | |
|----------|-------|---|--|
| 87E00235 | 41724 | F | No lesions recognized |
| 238 | 41726 | F | No lesions recognized |
| 240 | 41728 | F | No lesions recognized. Necropsied 16 Sept 1987 |
| 243 | 41723 | F | No lesions recognized |
| 246 | 41725 | F | No lesions recognized |
| 248 | 41727 | F | Ileum congestion with marked congestion of Peyer's Patches. Necropsied 16 Sept 1987. |
| 254 | 41721 | F | No lesions recognized. |
| 268 | 41722 | F | No lesions recognized. |

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