CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-264

Pharmacology Review(s)

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA number	21-264
Review number	2
Sequence number/date/type of submission	October 17, 2003
Information to sponsor Yes (X) No ( )	
Sponsor and/or agent	Bertek Pharmaceuticals Inc
Manufacturer for drug substance	Draxis Pharma Inc , Kirkland, Quebec, Canada

Reviewer name	Paul Roney, Ph D , D A B T
Division name	Neuropharmacological Drug Products
HFD #	120
Review completion date	March 8, 2004

Drug

Trade name Apokyn

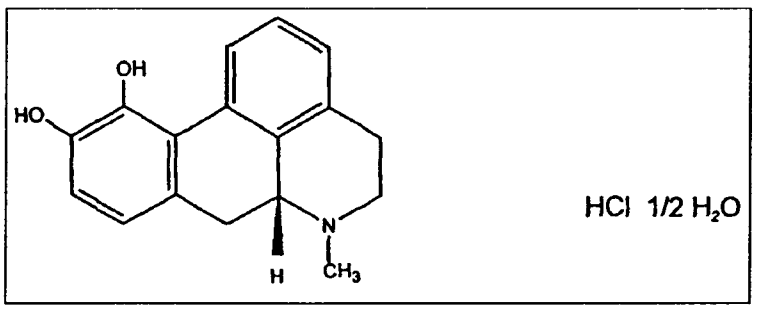
Generic name (list alphabetically) Apomorphine

Chemical name 4H-Dibenzo[de,g]quinoline-10,11-diol, 5,6,6a,7-tetrahydro-6-methyl-, hydrochloride, hemihydrate

CAS registry number 41372-20-7

Molecular formula/molecular weight $C_{17}H_{17}NO_2$ HCl 1/2H_2O / 312 79

Structure



Relevant INDs/NDAs IND 52,844

Drug class Dopamine Agonist

Indication Parkinson's Disease

Clinical formulation

Vial -10 mg/ml apomorphine and 1 mg/ml sodium bisulfite

Cartridge- 10 mg/ml apomorphine, 1 mg/ml sodium bisulfite and 5 mg/ml benzyl alcohol

Route of administration Subcutaneous Injection 2-6 mg/dose up to five doses/day

Disclaimer Tabular and graphical information from sponsor's submission are identified as such

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EXECUTIVE SUMMARY

The sponsor (Bertek Pharmaceuticals) is seeking approval of apomorphine for the treatment of acute off episodes in Parkinson's disease The Division issued an Approvable Letter on July 2, 2003 In the Approvable Letter, the Division identified seven preclinical issues for the sponsor to address (see Appendix 1, page 13) Of these seven issues, three (carcinogenicity studies in mice and rats, reproductive toxicity battery, and in vivo micronucleus mouse test) were Phase IV issues In this submission, the sponsor has committed to fulfilling these requests post approval A timeline for completion of these studies needs to be determined

A fourth issue (mass balance studies in mice, rats and monkeys) was initially designated as a pre-Approval issue, but was subsequently changed to a Phase IV commitment (see Appendix 2, page 14) The sponsor has committed to conducting a mass balance study in rats The sponsor requested that the requirement for mass balance studies in mice and monkeys be dropped The sponsor argues that since they have not conducted studies in mice, there is no need for a mouse mass balance study However, as noted in the previous paragraph, the sponsor has committed to conducting a carcinogenicity study in mice A mass balance study is needed to assess the implications of this study for humans This reviewer recommends that the requirement for a mouse mass balance study be retained The sponsor also requested that the requirement for the monkey study should be dropped They argue that a mass balance study (— Project 152024) submitted in the original NDA fulfilled this requirement This study was reviewed on page 9 of the original NDA review (where it was listed as — Report 8962) and found inadequate The plasma levels of radioactivity were too low to permit identification of metabolites, which is the primary reason for requesting these studies This reviewer recommends that the requirement for a monkey mass balance study be retained

The fifth issue to resolve was the need for a hERG channel assay The sponsor has conducted the hERG channel assay and a dog purkinje fiber assay (which was not requested by the division) Apomorphine inhibit the hERG channel with an IC50 of 0 127 uM No effects on Action Potential Duration in dog purkinje fibers were noted at doses up to 1 uM Higher doses should have been used in this study The sponsor also submitted a review of the cardiovascular effects of apomorphine by a consultant (— A portion of his review (Section 6) was essentially identical to the original Bertek review dated March 2, 2002 (pages 5-1-95 to 5-1-97) Using a comparison of the levels causing hERG channel prolongation and clinical apomophine concentrations, it was concluded that apomorphine has minimal potential for prolonging the QT interval in patients However, it is not acceptable to compare in vivo and in vitro concentrations in this manner It is the FDA and ICH position that a positive test result in an assay, such as the hERG assay, raises concerns about potential QT prolongation that requires careful study in humans

The sixth issue to resolve was the CMC specifications for certain impurities were above the level of qualification The sponsor was requested to either lower the CMC specifications or conduct toxicological studies to qualify the impurities The sponsor has lowered the specifications for the impurities so that they are below the level of qualification This issue is considered resolved

The final issue was for the sponsor to review the toxicity of Tigan Tigan is an anti-emetic taken to treat the nausea induced by apomorphine Tigan is only taken during the first few months of therapy with apomorphine The sponsor has submitted a summary of available data on Tigan Only limited data are available on the preclinical toxcity of Tigan The sponsor concluded that the available data do not support or preclude long-term dosing in humans at

therapeutic dose levels This reviewer agrees that the available data do not suggest any serious concerns about the use of Tigan However, the data are inadequate to support the use of Tigan Specifically, there are no repeat dose oral toxicity studies or genotoxicity studies available on this compound

If the sponsor agrees to these recommendations, then the preclinical studies are adequate support the Approval of this application

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SAFETY PHARMACOLOGY-CARDIOVASCULAR

Effects of Dopamine, Apomorphine, and Ropinirole on Cloned hERG Channels expressed in Mammalian Cells

Bertek Study Report #018-002
The effect of apomorphine (0 03, 0 1, 0 3 and 1 uM) on the hERG channel in transfected HEK293 cells was examined Haloperidol was the positive control

Apomorphine Inhibition of hERG current

Concentration (uM)	N	Mean Inhibition	SEM
0 03	3	17 6%	1 0%
0 1	3	49 4%	1 5%
0 3	6	67 0%	5 8%
1 0	3	91 5%	3 0%

The IC50 was 0 127 uM

100 uM Dopamine did not significantly alter the hERG channel
The IC50 for ropinirole was 1 214 uM
At 0 1 uM, haloperidol inhibited the hERG channel by 91 6%

Effects of Apomorphine, Dopamine and Ropinirole on Action Potential in Isolated Canine Cardiac Purkinje Fibers

Bertek Study Report #018-003

The effect of apomorphine (0 01, 0 1 and 1 uM) on isolated dog purkinje fiber cells was examined Sotalol (100 uM) was the positive control

Table 1 Summary of the Effects of Apomorphine on Action Potential Parameters at 2 s, 1s and 0.5s BCL's

2 s BCL					
	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
Concentration	Mean± S E M	Mean± S E.M	Mean± S E.M	Mean± S.E M	Mean± S.E M
0 01 μM	-6 2 ± 6 9	-4 5 ± 6 4	-2 8 ± 3 1	4 6 ± 6 9	15 8 ± 9 1
0 1 μM	-11 7 ± 6 3	-9 8 ± 5 5	-1 8 ± 2 0	4 7 ± 6 5	6 0 ± 8 2
1 μM	-0 5 ± 6 0	0 0 ± 4 8	-1 5 ± 1 2	3 0 ± 5 2	3 6 ± 8 3

1s BCL					
	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
Concentration	Mean±	Mean±	Mean± S E M	Mean± S E M	Mean± S E M
0 01 μM	-0 6 ± 3 9	-0 3 ± 4 2	-3 2 ± 3 3	6 5 ± 8 1	13 0 ± 14 5
0 1 μM	-7 9 ± 3 4*	-6 7 ± 3 4	-1 7 ± 2 1	7 1 ± 8 3	12 7 ± 11 7
1 μM	1 0 ± 4 9	1 4 ± 4 0	-2 9 ± 2 5	8 1 ± 8 4	12 9 ± 12 6

0 5 s BCL					
	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
Concentration	Mean±	Mean±	Mean± S E.M	Mean± S E.M	Mean± S E.M
0 01 μM	-1 2 ± 2 5	0 7 ± 2 9	-3 8 ± 4 0	9 5 ± 8 8	18 1 ± 13 2
0.1 μM	-5 3 ± 1 6	-4 0 ± 1 9	-2 5 ± 2 8	9 0 ± 8 7	17 7 ± 10 1
1 μM	-0 2 ± 2 8	2 0 ± 2 9	-3 7 ± 3 0	10 1 ± 8 9	19 8 ± 13 8

BCL, Basic Cycle Length, APD_{60} and APD_{90}, action potential duration measured at 60% and 90% repolarization, Δ%, Percent change from baseline values, ΔmV absolute change from baseline in millivolts, NA, not applicable, RMP, resting membrane potential, APA, action potential amplitude, Vmax, maximum rate of depolarization * Denotes statistical significance ($p<0.05$) when compared to the vehicle control sequence

Figure 1, from page 22 of Report 018-003

Table 4 Summary of the Effects of Vehicle control on Action Potential Parameters at 2 s, 1s and 0.5s BCL's

Vehicle Control Sequence	2 s BCL				
	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
	Mean± S E M	Mean± S E M	Mean± S E.M	Mean± S.E.M	Mean± S.E.M
1	-0 5 ± 0 6	-1 1± 0 8	2 1 ± 1 3	2 5 ± 2 5	8 9 ± 12 6
2	3 9 ± 1 9	2 2 ± 1 2	-2 5 ± 0 8	0 5 ± 1 3	1 3 ± 6 1
3	5 1 ± 2 9	4 1 ± 2 0	2 4 ± 0 8	7 3 ± 4 1	24 5 ± 18 9

Vehicle Control Sequence	1 s BCL				
	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
	Mean± S E.M	Mean± S E.M	Mean± S E.M	Mean± S E.M	Mean± S E M
1	-1 5 ± 1 2	-1 4 ± 0 7	-0 7 ± 1 0	-1 6 ± 4 1	5 6 ± 13 0
2	1 7 ± 1 4	0 5 ± 0 9	-2 0 ± 0 9	-0 4 ± 1 1	-2 5 ± 6 1
3	3 7 ± 2 3	2 8 ± 2 1	2 1 ± 0 8	5 7 ± 3 4	13 3 ± 7 0

Vehicle Control Sequence	0 5 s BCL				
	APD_{60}, Δ%	APD_{90}, Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
	Mean± S.E M	Mean± S E.M	Mean± S.E.M	Mean± S E M	Mean± S E.M
1	-0 6 ± 2 9	-0 5 ± 2 5	-0 7 ± 0 7	-1 5 ± 3 7	-2 0 ± 17 1
2	0 6 ± 1 9	0 2 ± 1 6	-1 0 ± 1 0	1 8 ± 1 3	7 6 ± 7 2
3	3 1 ± 2 1	2 5 ± 1 7	-1 7 ± 0 8	6 0 ± 3 3	12 0 ± 5 0

BCL, Basic Cycle Length, APD_{60} and APD_{90}, action potential duration measured at 60% and 90% repolarization Δ% Percent change from baseline values ΔmV, absolute change from baseline in millivolts, NA, not applicable, RMP, resting membrane potential APA, action potential amplitude, Vmax, maximum rate of depolarization.

Table 5 Summary of the Effects of 100 μM *dl*-sotalol on Action Potential Parameters

	APD_{60}, Δ%	APD_{90} Δ%	RMP, ΔmV	APA, ΔmV	Vmax, Δ%
BCL (s)	Mean± S E.M	Mean± S E.M	Mean± S E.M	Mean± S.E.M	Mean± S.E M
2	69 5 ± 22 2*	67 5 ± 22 8 *	1 3 ± 1 3*	10 7 ± 11 2	-19 7 ± 19.2
1	49 1 ± 9 3 *	50 6 ± 11 8 *	1 4 ± 1 8*	4 9 ± 5 4	21 1 ± 18 9
0 5	28 7 ± 2 7	30 8 ± 4 3 *	2 7 ± 1 9	1 3 ± 5 0	-27 7 ± 17 5

BCL, Basic Cycle Length, APD_{60} and APD_{90}, action potential duration measured at 60% and 90% repolarization, Δ%, Percent change from baseline values, ΔmV, absolute change from baseline in millivolts, NA, not applicable, RMP, resting membrane potential, APA, action potential amplitude, Vmax, maximum rate of depolarization * APD_{60} and APD_{90} were statistically ($p<0.05$) more prolonged when compared to the vehicle control sequence 3

Figure 2, from page 25 of Report 018-003

OVERALL SUMMARY AND CONCLUSIONS

Drug History

Parkinson's disease is a neurodegenerative disease characterized by bradykinesia, muscular rigidity, resting tremors and postural instability The disease can progress to a rigid akinetic state in which the patient is incapable of taking care of himself Pathologically, Parkinson's disease is characterized by a progressive loss of dopaminergic neurons in the substantia nigra resulting in decreased dopaminergic tone Since the cause of this loss is unknown at present, current therapy for Parkinson's disease utilizes substances that increase dopaminergic tone (e g , levodopa, ropinerole, pramipexole) or increase the amount of dopamine available at the receptor site (e g COMT inhibitors, MAO-B inhibitors) Despite the use of these drugs, patients may still experience "off" episodes in which their muscle movement is slow or frozen These off episodes are thought to be the result of deficient dopaminergic tone

Apomorphine is a dopamine agonist which was discovered in the 19th century Due in part to its rapid metabolism, it has not been used in the United States for maintenance treatment of Parkinson's disease The sponsor is proposing that subcutaneously injected apomorphine would be a safe and effective treatment of acute off states in patients with Parkinson's disease The sponsor submitted their initial application on January 2, 2003 The FDA issued an Approvable Letter on July 2, 2003 Seven preclinical issues were raised in this Letter (see page 13) The sponsor submitted its response to these issues on October 17, 2003 In addition, revised label language was submitted on December 23, 2003

Cardiovascular Issues

At the time of sponsor's original NDA submission (May 6, 2002), the sponsor had not conducted any preclinical cardiovascular safety pharmacology studies Instead, the sponsor submitted reports from the open scientific literature to address this issue This reviewer reviewed these studies and concluded that the studies were inadequate to determine whether apomorphine had the potential to affect ECG parameters This reviewer did not recommend that cardiovascular safety pharmacology studies be conducted since he believed that there was sufficient clinical data to obviate the need for additional preclinical studies In subsequent discussions, it became evident that the clinical data were not sufficient to address this concern The sponsor was therefore asked to conduct a hERG channel assay prior to approval to determine whether apomorphine has the potential to affect the QT interval In this submission, the sponsor submitted studies on the hERG channel and dog Purkinje fibers The sponsor also submitted a report by ——— summarizing the cardiovascular studies conducted to date on apomorphine

Apomorphine inhibited the hERG channel with an IC50 of 0 127 uM There was approximately 90% inhibition at 1 uM apomorphine This indicates that apomorphine has the potential to prolong the QT interval

In contrast, apomorphine did not prolong the action potential duration in dog Purkinje cells at concentrations up to 1 uM Higher doses should have been used to more fully examine apomorphine's potential to affect the action potential in this system The positive control (dl-soltalol) inhibited the action potential while the vehicle control had no effect on the action potential

Dr ——— reviewed the available literature on the cardiovascular effects of apomorphine Most of the studies had been previously submitted Indeed, a portion of his review (section 6) is virtually identical to the original Bertek review dated March 2, 2002 (pages 5-1-95 to 5-1-97) He did refer to an unpublished study using isolated perfused guinea pig heart preparation His results indicate that the heart rate was slowed at 10 uM and the QTc(F) was prolonged at 100 uM, but the data were not submitted In the absence of the actual data, it is impossible to judge the adequacy and interpretation of these data Dr ——— also compares the in vitro concentrations which inhibit the hERG channel to the clinical apomophine concentrations He concludes that there is an adequate margin of safety (four-fold) between the in vitro concentration and the potential clinical concentration to that there is a minimal risk of QT prolongation in patients However, it is not appropriate to compare in vivo and in vitro concentrations in this manner In vitro studies indicate whether there is potential for a problem (hazard identification), but the results should not be directly compared to plasma levels

In conclusion, apomorphine inhibits the hERG channel, but did not affect the action potential duration in dog Purkinje fibers at doses up to 1 uM The guidelines state that "Any non-antiarrhythmic pharmaceutcal that blocks repolarizing ionic currents in nonclinical sutdies should be considered to pose a risk to humans "[1] It is concluded that apomorphine has the potential to affect the QT interval The clinical reviewer will need to determine whether a clinical study will be needed to assess the potential effects of apomorphine on the QT interval

[1] ICH Steering Committee (2002) Safety Pharmacology Studies for Assessing the Potential for Delayed Ventricular Repolarization (AT Interval Prolongation) by Human Pharmaceuticals [DRAFT] Page 6

Sponsor Response to Phase IV Commitments

The Approvable letter identified three sets of studies for the sponsor to perform as Phase IV commitments (carcinogenicity studies in mice and rats, reproductive toxicity battery, and in vivo micronucleus mouse test) The sponsor has committed to fulfilling these requests post approval, although they did not identify a timeline for fulfilling these commitments

Mass balance studies in mice, rats and monkeys were initially designated as a pre-Approval issue, but was subsequently changed to a Phase IV commitment (see Appendix 2, page 14) The sponsor has committed to conducting a mass balance study in rats The sponsor requested that the requirement for mass balance studies in mice and monkeys be dropped The sponsor argues that since they have not conducted studies in mice, there is no need for a mouse mass balance study However, as noted in the previous paragraph, the sponsor has committed to conducting a carcinogenicity study in mice A mass balance study is needed to assess the implications of this study for humans This reviewer recommends that the requirement for a mouse mass balance study be retained The sponsor also requested that the requirement for the monkey study should be dropped They argue that a mass balance study (— Project 152024) submitted in the original NDA fulfilled this requirement This study was reviewed on page 9 of the original NDA review (where it was listed as — Report 8962) and found inadequate The plasma levels of radioactivity were too low to permit identification of metabolites, which is the primary reason for requesting these studies This reviewer recommends that the requirement for a monkey mass balance study be retained

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Other Issues-Impurities, Tigan and Label

One issue to resolve was the CMC specifications for certain impurities were above the level of qualification The sponsor was requested to either lower the CMC specifications or conduct toxicological studies to qualify the impurities The sponsor has lowered the specifications for the impurities so that they are below the level of qualification This issue is considered resolved

The sponsor was asked to evaluate the preclinical studies on Tigan Tigan is an anti-emetic which is approved for acute use in the United States for the treatment of peri-operative nausea and nausea associated with gastroenteritis Since apomorphine is an emetic, it is recommended that patients taking apomorphine also take an anti-emetic, such as Tigan, until the patients adapt to apomorphine This could take two to three months The sponsor submitted a review by —— (Independent Consultant in Toxicology) Only limited data were available The only oral toxicity data available were reproductive toxicity studies in which rats were exposed through two mating cycles In these studies, rats were administered up to 100 mg/kg/day without any adverse effects on reproductive outcome Multiple dose studies using intravenous or intramuscular administration in rats (IM only), rabbits (IV only), dogs and monkeys did not identify any signs of toxicity The high dose in the IV studies were 30 mg/kg and 100 mg/kg in the IM studies The sponsor concluded that the limited data do not support or preclude long-term dosing in humans at therapeutic dose levels This reviewer agrees that the available data do not raise any major concerns about the use of Tigan However, the available data are inadequate to support the longer term use of Tigan Specifically, there is a lack of repeat dose toxicity studies and genotoxicity studies on Tigan

This reviewer has examined the revisions to the proposed labeling submitted by the sponsor on December 23, 2003 The sponsor has incorporated the Agency's recommended changes to the preclinical section of the label This reviewer has no changes to recommend to the present label

RECOMMENDATIONS

It is recommended that the sponsor be asked to commit to conducting mass balance studies in monkeys and mice as recommended in the original Approvable Letter In addition, the sponsor should commit to a timeline to completing the recommended Phase IV commitments in a timely manner Provided the sponsor agrees to these commitments, the available preclinical data are sufficient to approve this NDA

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APPENDIX 1: PHARMACOLOGY / TOXICOLOGY COMMENTS FROM THE APPROVABLE LETTER

PHARMACOLOGY / TOXICOLOGY

1 **You will need to assess the carcinogenic potential of apomorphine by conducting carcinogenicity studies in mice and rats post-approval. [redacted]**

2. **You will need to conduct and submit the reproductive toxicity studies specified in ICH guidelines, you may submit the results of these studies post approval**

3. **You have set specifications for two degradants that are above the threshold of qualification If you are unable to lower the specification for these products, you will need to conduct qualification studies of the degradants prior to approval of the drug product**

Figure 3, from Page 3 of the July 2, 2003 Approvable Letter

4 Prior to approval, you will need to conduct and submit results of an in vitro HERG channel assay in which you have examined effects of a wide range of apomorphine doses You should include positive and negative drug controls in each assay

5 As a phase 4 commitment, we ask that you repeat the in vivo micronucleus test using a multiple dosing regimen which would more closely resemble the intended clinical use

6. **You should determine the metabolism of apomorphine by conducting mass balance studies in mice, rats and monkeys; this should be done prior to approval.**

7 **Finally, we ask that you review the preclinical literature for information supporting the safety of Tigan administered chronically**

Figure 4, from Page 4 of July 2, 2003 Approvable Letter

APPENDIX 2: AUGUST 7, 2003 MEETING MINUTES

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Page 1

MEMORANDUM OF TELECON

DATE: August 7, 2003

APPLICATION NUMBER NDA 21-264 Apomorphine Injection

BETWEEN
Name: Andrea Miller
Dr O'Donnell
Dr Bottim
Dr Van Loom
Dr Smith
Dr Shaw
Phone (301) 594 6649
Representing: **Bertek Pharmaceuticals**

AND
Division of Neuropharmacological Drug Products HFD 120
Name Dr Katz - Division Director
Dr Feeney – Group Leader
Dr Kapcala – Medical Reviewer
Dr Freed – Pharmacology Team Leader
Dr Roney – Pharmacology Reviewer
Dr Uppoor – Clinical Pharmacology & Biopharmaceutics Team Leader
Dr Dunn – Clinical Pharmacology & Biopharmaceutics Reviewer
CDR Teresa Wheelous – Sr Regulatory Management Officer

SUBJECT Timing of Mass Balance Studies Requested in the Approvable Letter

BACKGROUND:
In a July 11, 2003 submission, the sponsor requested a telecon to discuss the Toxicology and Clinical Pharmacology requests for mass balance studies prior to approval as stated in the Agency's July 2, 2003 approvable letter Bertek Pharmaceuticals provided posters along with an argument in an August 1, 2003 submission in support of their position to conduct the mass balance study post approval.

Additionally, this August 1, 2003 submission requested guidance on (1) the Agency's acceptance of an algorithm to use in defining a specific off as either an end-of-dose or a spontaneous off, and (2) acceptance of December 31, 2002 as the new cut-off date for the safety update and June 30, 2003 as the cut-off date for serious adverse events

DISCUSSION:
Mass Balance Study

- Bertek stated that the completion of the mass balance study would be from 6- 9 months in total. Six to 12 weeks is required to develop the isotope and about 6 months to conduct the study

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- **Bertek expects to completely reply to the approvable letter in late September 2003 if the Division agrees that the mass balance studies data can be provided at a later date.**

- **Previously, Bertek believed that, based on old and faulty techniques, auto-oxidation is the major metabolic elimination path, but currently Bertek no longer believes that auto-oxidation is the major metabolic pathway**

Use of Proposed Algorithm for Characterizing Treatment of "Off" Relative to Dosing Interval

- **The Division had questions about proposed algorithm**

- **There was some discussion about defining end of dose "off" relative to the length of the dosing interval and not just in terms of absolute time (i e., one hour pre next dose)**

Cut-off Date Changes to Safety Update and Serious Adverse Event

- The Division accepts the change in the cut-off date for the safety update from May 31, 2002 to December 31 2002 and accepts the new Serious Adverse Event report cut-off date June 30, 2003

ACTION ITEMS

1 Bertek will provide as much information as available regarding plasma metabolite data, and an argument explaining why auto-oxidation is incorrect and why the studies represented in the posters are accurate

2 Since a mass balance study will take almost a year to conduct, it may be acceptable to accept these data post approval Bertek should provide a time line for completion of this study.

3 The Division will present this concern to the Office Director, Dr Temple.

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/s/

Paul Roney
3/31/04 01 38 42 PM
PHARMACOLOGIST

Lois Freed
3/31/04 02 10 10 PM
PHARMACOLOGIST

MEMORANDUM

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

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

Date 6/18/2003

From Lois M Freed, Ph D
Supervisory Pharmacologist

Subject NDA 21-264

The nonclinical data submitted in support of NDA 21-264 (apomorphine HCl s c) were reviewed by Paul Roney, Ph D , D A B T (Pharmacology/Toxicology Review and Evaluation, 6/18/2003) Nonclinical studies included the following limited PK/ADME data, general toxicology (13- and 26-week s c studies in Sprague-Dawley rat, 13-week combination [apomorphine + levodopa/carbidopa] study in Sprague-Dawley rat, 13- and 39-week s c studies in cynomolgus monkey), genetic toxicology (Ames, *in vitro* mouse lymphoma, *in vitro* chromosomal aberration in human lymphocytes, *in vivo* micronucleus assay in CD-1 mouse, *in vivo/in vitro* UDS in Wistar rat) No reproductive and developmental toxicology or carcinogenicity studies were conducted, the sponsor included a request for a waiver of requirements for these studies in the NDA submission It is Dr Roney's recommendation that the NDA not be approved based on the lack of reproduction and carcinogenicity studies

The sponsor's request for waiver of carcinogenicity study requirements was based on the seriousness of the indication, the average age of the patient population (>65 yrs), the relatively short duration of treatment (5-6 yrs), and the lack of positive findings in the *in vivo* genotoxicity assays The sponsor also noted that "at least four" carcinogenicity studies have already been conducted (26-week study in p53+/- heterozygous mouse, 18-month study in mouse, 22-month study in male rats, 23-month study in female rats), and that three of these studies have been reviewed by the FDA and presented at an advisory committee meeting (Advisory Committee Meeting of the Urology Subcommittee for Reproductive Health Drugs, April 10, 2000, transcripts and briefing material are available to the public via the CDER internet site) Dr Roney did not find the sponsor's basis for a waiver compelling and recommended that the sponsor conduct carcinogenicity studies in mouse and rat prior to approval

The lack of positive findings in the *in vivo* genotoxicity assays, the anticipated duration of treatment (5-6 yrs), and average age of the patient population are not compelling reasons for a waiver of carcinogenicity studies In the waiver request, the sponsor noted the lack of positive findings in the *in vivo* genotoxicity studies, but did not discuss the reproducible positive findings (in the absence and presence of metabolic activation) in numerous *in vitro* genotoxicity studies (i e , Ames, mouse lymphoma, chromosomal aberration in human lymphocytes) The lack of positive findings in the *in vivo* genotoxicity studies does not diminish the concern regarding the

positive *in vitro* findings or concern regarding carcinogenic potential The sponsor's anticipated duration of treatment (5-6 yrs) is consistent with the need for carcinogenicity studies According to the ICH S1A guidance (Guidance for Industry The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals ICH S1A March 1996), carcinogenicity studies are needed for pharmaceuticals intended to be used chronically for ≥6 mo or to be used intermittently for a chronic indication (e g , depression, anxiety) The guidance does state that a short life-expectancy may be a reason for waiving the requirement for carcinogenicity studies, however, "short" is defined as " less than 2 to 3 years " Granting a waiver solely on the basis of the (potentially) advanced age of the patient population would not be consistent with current practice within the Division since carcinogenicity studies are routinely required prior to approval for drugs intended to treat Alzheimer's disease, an indication almost exclusively involving older patients

Dr Roney is correct in pointing out that the

the following carcinogenicity studies were conducted 25-wk s c study in p53+/- transgenic mice, 22-month s c study in male rats, 24-month s c study in female rats In the p53+/- study

no increase in the incidence of systemic tumors were observed in apomorphine-treated animals The primary tumor finding was subcutaneous sarcomas at the injection site The incidence was 0/30, 22/30, 27/30, 26/30, and 24/30 in untreated C, vehicle C, LD, MD, and HD, respectively concluded that the finding was not considered drug-related due to the similar incidence in vehicle C animals, and published studies suggesting an increased susceptibility of p53+/- transgenic mice to sarcomas of the skin "at the site of chronic mild irritation" (As noted Blanchard *et al* [Blanchard KT *et al* *Toxicol Path* 27(5) 519-527, 1999] reported an increase in subcutaneous sarcomas in heterozygous p53+/- transgenic mice at the site of implantation of radio transponder identification devices)

According the 22-month study in male Sprague-Dawley rat was conducted using doses of 0, 0 1, 0 3, 0 8, and 2 0 mg/kg/day s c Dosing was discontinued in the high-dose group at Wk 26 due to " excessive behavioral alterations ", high-dose animals remained on-study and were sacrificed at Wk 97, when all groups were sacrificed due to excessive mortality in the vehicle C group There was a significant increase in skin (injection site) fibromas and testicular interstitial cell adenomas The interstitial cell tumors were considered "secondary to hormonal imbalance (decreased prolactin, increased LH)", however, the hormone data were not completely consistent with this mechanism The data are summarized below

Neoplastic Findings in Apomorphine Treated Male Rats vs. Vehicle control

Tissue/Finding	Vehicle Gp 2	0 1 mkd Gp 3	0 3 mkd Gp 4	0 8 mkd Gp 5	P-value
Pituitary , Adenoma	42	47	41	31**	P < 0 001
Skin/fibroma	3/70	1/69	2/70	7/69	P < 0 03
Testes/ Interstitial cell tumor	0/70	0/69	3/69	9/70	P < 0 001

Neoplastic Findings in Apomorphine Treated Male Rats vs Untreated control

Tissue/Finding	Untreated Gp 1	0 1 mkd Gp 3	0 3 mkd Gp 4	0 8 mkd Gp 5	P-value
Skin/fibroma	1/68	1/69	2/70	7/69	P < 0 005
Testes/ Interstitial cell tumor	3/70	0/69	3/69	9/70	P < 0 003

The 24-month study in female Sprague-Dawley rat was conducted using doses of 0 1, 0 3, 0 8, and 2 mg/kg/day s c Dosing of the LD group was discontinued prior to the 6th month without evaluation The study was terminated during Wk 100 due to excessive mortality in the vehicle C group The incidence of injection site (subcutaneous) sarcoma was significantly increased at the high dose The data were summarized in the following table

Neoplastic Findings in Apomorphine Treated Female Rats vs Vehicle control

Tissue/Finding	Vehicle Gp 2	0 3 mkd Gp 3	0 8 mkd Gp 4	2 mkd Gp 5	P-value
Mammary Adenoma	3	4	10*	5	P < 0 05
Skin/ subcutaneous sarcoma	1	0	3	6 **	P <0 002

According to review, the concentration of apomorphine used in the female rat carcinogenicity study was 1 mg/mL, the concentration of apomorphine in the to-be-marketed formulation is 10 mg/mL Therefore, there is no margin of safety between the concentration used in the female rat and that to be administered to humans

In addition to the studies in p53+/- transgenic mice and male and female rats, the sponsor noted that an 18-mo carcinogenicity study in mouse has been conducted However, no review of, or reference, to an 18-month mouse carcinogenicity study of apomorphine was found

The significant increase in injection site sarcomas was dismissed during the reviewing NDA — because the clinical route of administration was sublingual, not subcutaneous However, for this NDA the clinical route is subcutaneous and, therefore, this finding is clinically relevant These data, in conjunction with the positive findings in the *in vitro* genotoxicity assays (Ames, mouse lymphoma, chromosomal aberration in human lymphocytes) in the absence and presence of metabolic activation, increase the concern regarding the carcinogenic potential of apomorphine

If the tumor finding (i e , injection site sarcomas) observed in carcinogenicity study in female rats is allowed to be included in labeling for Bertek's product, then it is recommended that the mouse and rat carcinogenicity studies be conducted as a Phase 4 commitment, with a definite timetable for completion and full study submission However, if the tumor finding cannot be referred to in labeling, then it is recommended that the carcinogenicity studies be conducted prior to approval

(In addition to the tumor finding in the carcinogenicity study in female rats —— — there was an increase in retinal atrophy in males at doses of 0 1-0 8 mg/kg and in females at doses of 0 3-2 0 mg/kg This finding has been reported with other dopamine agonists Dr Feeney requested that the retinal finding be included in the safety evaluation of Bertek's NDA, therefore, it has been included in the proposed labeling)

Reproduction studies

The sponsor's basis for waiver of reproduction study requirements is as follows (a) "the seriousness of the disease state that is intended to be treated (i e , late stage Parkinson's disease)", (b) "the age and reproductive capacity of the patient population for which this drug is to be indicated", the "approximated age of the patient population is greater than 73 years old", (c) "previous literature that reports the findings of reproductive studies (Segment I) performed with apomorphine via the subcutaneous route", (d) the lack of positive findings in a number of *in vivo* genotoxicity studies (i e , " mouse micronucleus and rat unscheduled DNA synthesis tests ") "at concentrations approximately 35 times greater than the maximum possible systemic dose in human based on a mg/kg comparison"

The sponsor noted that the approximate age of the intended patient population is >73 years and that "The debilitating nature of late-stage Parkinson's disease " would severely limit sexual activity In a brief summary of the results of the published Segment I study, the sponsor pointed out that "No adverse effects were noted" on a number of reproductive parameters, including reproductive organ weight (i e , testicular, epididymal and prostate), sperm assessments fertility indices or on fetal viability "

Dr Roney did not find the sponsor's basis for a wavier of reproduction studies compelling He noted the following (a) reproduction studies have been required prior to approval for other drugs for serious indications (e g , AIDS, cancer, organ rejection), reproduction studies were required for COMTAM®, a drug "indicated for prevention of end of dose "off" episodes, (b) reproduction studies have been waived for drugs belonging to a class known to produce "severe effects on the developing embryo/fetus", however, this does not apply to apomorphine, (c) the intended patient population is not limited to individuals without reproductive potential, (d) the published Segment I study does not provided sufficient information to allow an adequate review and the study report is not in the public domain (e) the results of the *in vivo* genotoxicity studies are not an adequate substitute for reproduction studies

Dr Roney noted that "The age of onset for Parkinson's disease is between 20 and 80 " and " the minimum ages in [the sponsor's] four clinical trials were 38, 42, 45, and 46 years of age" Dr Roney also noted that the sponsor's clinical database included patients who were not severely affected (i e , " the sponsor studies inclusion criteria include Hoehr-Yahr stage of at least 2") and that " new therapies are available for treatment of sexual dysfunction thus, allowing the reproductively active period to be extended Dr Roney recommended that the sponsor submit a full battery of reproduction studies prior to approval or

Regarding the issue of the need for reproductive toxicology studies, the sponsor's basis for waiver of these studies is similar to that for a waiver of carcinogenicity studies However, the sponsor emphasized the relatively advanced age of the patient population that, in conjunction with the severity of the disease state, would severely limit the potential for reproduction The sponsor did cite a published Segment I study (Yousseff *et al Toxicol Sci* 51 273-279, 1999),

however, as correctly noted by Dr Roney, a published study does not usually (as in this case) provide sufficient information to allow for an adequate review of the data (Also as noted by Dr Roney, the sponsor does not have [redacted] and the report is not in the public domain) Even if adequate, a Segment I (i e , mating and fertility) study would not constitute a complete assessment of drug-related effects on reproductive parameters

(In the published mating and fertility study [Yousseff *et al* , 1999], there appeared to be a decrease in the fertility index in male rats treated subcutaneously with apomorphine at a dose of 2 mg/kg Although this published report did not provide sufficient detail [e g , individual line listings] to allow for an adequate review, the effect on the fertility index should be included in labeling)

Dr Roney disagreed with the sponsor's assessment of the reproductive potential of the intended patient population The sponsor estimates the "approximated age" of the target population for apomorphine to be 73 yrs However, data provided by the sponsor at the request of the FDA (e-mail, letter date 5/28/03) indicated that the mean age of all patients treated with apomorphine in clinical trials was 64-65 yrs (range 38-99 yrs, 25th percentile 57-58 yrs) According to Dr Roney, at least four patients were <50 yrs of age (i e , 38, 42, 45, and 46 yrs) It has been estimated that as many as 10% of PD patients have onset of symptoms prior to age 40 yrs (Hoehn MMM *Neurology Clinics* 10(2) 331-339, 1992), and that on-off episodes may begin within a few years of onset of symptoms or initiation of treatment with L-dopa (e g , Shoulson I *et al Ann Neurol* 44(Suppl 1) S160-S166, 1998) (The indication described in the sponsor's package insert for apomorphine does not limit the age of the patient population)

Of the anti-PD medications marketed, COMTAN® would appear to have an indication most similar to that proposed for apomorphine COMTAN® (NDA 20-796, approved 10/19/99) was approved as " an adjunct to levodopa/carbidopa to treat patients with idiopathic Parkinson's Disease who experience the signs and symptoms of end-of-dose "wearing-off" The labeling for COMTAM® further states that "Comtan's effectiveness has not been systematically evaluated in patients with idiopathic Parkinson's Disease who do not experience end-of-dose 'wearing-off '" The age range of PD patients treated in controlled and uncontrolled clinical trials submitted in support of the NDA (20-796) was ≈30-80 yrs (mean ≈ 65 yrs) Of those exposed to long-term treatment, 55% were <65 and 45% were >65% (according to the clinical review of NDA 20-796) This proportion was considered representative of the intended patient population It would seem, therefore, that PD patients with "off" episodes (i e , patients who might be treated with apomorphine) may include individuals within a fairly wide age range [Two-year carcinogenicity studies in mice and rats and a complete battery of reproduction studies were required prior to approval for COMTAN®]

Based on the available information, it is recommended that the sponsor conduct a full battery of reproduction studies (cf Guideline for Industry--Detection of Toxicity to Reproduction for Medicinal Products, ICH-S5A Sept 1994) for apomorphine It is also recommended that these studies be conducted prior to approval, unless it is determined that apomorphine fulfills an unmet clinical need that warrants approval without data on potential reproductive toxicity If reproduction studies are to be conducted phase 4, it is recommended that a deadline be set for completion of the complete battery of studies

Other Issues

Dr Roney concluded that the sponsor did not provide adequate data on the metabolism of apomorphine in the animal species used in the definitive nonclinical safety studies (i e , rat, monkey, mouse) Circulating levels of parent compound and/or metabolites were not quantitated in the *in vivo* PK/ADME studies conducted by the sponsor (Plasma exposure to parent compound was quantitated in the 13-wk s c toxicity studies in rat and monkey, but not in either of the chronic toxicity studies In the 13-wk study in rat, only one time point was sampled [i e , no AUC data] No PK/ADME/TK data were provided for mouse) There would also appear to be a lack of metabolism data in humans, in particular a lack of data on the major circulating metabolites Without these data is difficult to assess the relevance of the animal models for assessing human risk The sponsor was informed during a pre-NDA meeting (12/10/99) that there was a " need for adequate data on the pharmacokinetics and metabolism of apomorphine in the animal species used for toxicity testing " Dr Roney concluded that "mass balance studies in mice, rats and monkeys" may be conducted Phase 4 However, considering the importance of these data in the interpretation of the nonclinical studies, it is recommended that quantitative data on parent compound and major circulating metabolites in the nonclinical animal species used in the definitive safety studies and in human be submitted prior to approval

I concur with Dr Roney's recommendation regarding the two degradants in the drug product [ ] and the need to repeat (Phase 4) the *in vivo* micronucleus assay in mouse and the 13-wk combination toxicity study in rats

Recommended labeling

[

]

CLINICAL PHARMACOLOGY

Mechanism of Action

[

]

1 pages redacted from this section of the approval package consisted of draft labeling

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA number	21-264
Review number	1
Sequence number/date/type of submission	May 6, 2002, April 1, 2003
Information to sponsor Yes (X) No ( )	
Sponsor and/or agent	Bertek Pharmaceuticals Inc
Manufacturer for drug substance	Draxis Pharma Inc , Kirkland, Quebec, Canada

Reviewer name	Paul Roney
Division name	Neuropharmacological Drug Products
HFD #	120
Review completion date	June 16, 2003

Drug

Trade name —

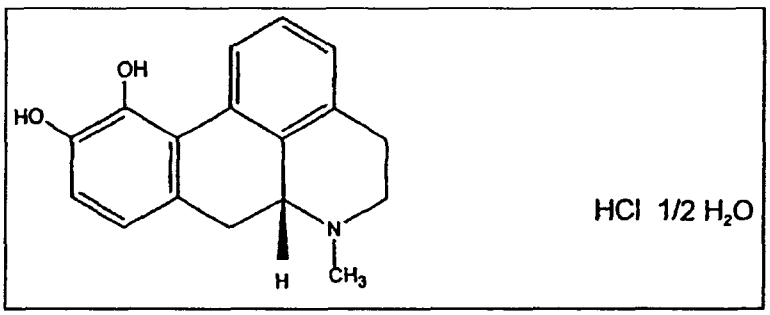
Generic name (list alphabetically) Apomorphine

Chemical name 4H-Dibenzo[de,g]quinoline-10,11-diol, 5,6,6a,7-tetrahydro-6-methyl-, hydrochloride, hemihydrate

CAS registry number 41372-20-7

Molecular formula/molecular weight $C_{17}H_{17}NO_2$ HCl 1/2H_2O / 312 79

Structure



Relevant INDs/NDAs IND 52,844

Drug class Dopamine Agonist

Indication Parkinson's Disease

Clinical formulation

Vial -10 mg/ml apomorphine and 1 mg/ml sodium bisulfite

Cartridge- 10 mg/ml apomorphine, 1 mg/ml sodium bisulfite and 5 mg/ml benzyl alcohol

Route of administration Subcutaneous Injection 2-6 mg/dose up to five doses/day

Disclaimer Tabular and graphical information from sponsor's submission are identified as such

Table of Contents

EXECUTIVE SUMMARY

The sponsor (Bertek Pharmaceuticals) is seeking approval of apomorphine for the treatment of acute off episodes in Parkinson's disease To support their application, the sponsor has submitted repeat toxicology studies (including chronic studies in rats and monkeys), genotoxicity studies, limited pharmacokinetic studies and local irritation studies The sponsor did not submit any studies on pharmacology, safety pharmacology, carcinogenicity or reproductive toxicity To address some of these data gaps, the sponsor submitted papers from the open scientific literature to provide data on pharmacology, safety pharmacology, pharmacokinetics and reproductive toxicity The data from the open literature is of limited utility in assessing the potential effects of apomorphine since it is not possible to conduct a detailed examination of the data In addition, there are substantive issues that have not been addressed in the open literature In particular, there is a lack of data on the potential effects of apomorphine on reproductive function and on the heart There is also a lack of data on the metabolism of apomorphine in preclinical species and on the carcinogenic potential of this genotoxic drug

Apomorphine is dopamine agonist which showed activity in several animal models of Parkinson's disease It has low oral bioavailability due to rapid metabolism It is therefore administered by subcutaneous injection The exact metabolic pathways have not been determined, although conjugation with glucoronic acid is an important pathway The metabolism of apomorphine has to be determined in preclinical species and compared to the human metabolism This will help to establish the relevance of the preclinical studies

The primary toxicity observed in apomorphine-treated animals (rats, monkeys) are clinical signs associated with excessive stimulation of dopamine receptors (hyperactivity, stereotypic behavior) Weight loss has been observed in treated animals, but no consistent effects on hematology or clinical chemistry parameters were observed One potential concern is male reproductive organs Decreased testes weight and altered testes histology were observed in rats and monkeys at doses comparable to what would be used clinically The potential effects on male reproduction have not been examined in segment I (mating and fertility) reproductive toxicity studies (see below) The sponsor also submitted a study examining the potential effects of Sinemet on apomorphine toxicity in rats, but the Sinemet doses were too low to permit meaningful comparisons This study will need to be repeated

Apomorphine is genotoxic in multiple in vitro systems It induced frameshift mutations in Ames assay, especially in TA1537 It was also positive in the mouse lymphoma assay causing an increase in both large colonies (indicative of mutations) and small colonies (indicative of clastogenic events) Apomorphine induced chromosomal aberrations in cultured human lymphocytes Apomorphine was negative in the in vivo mouse micronucleus test However, this test only used dosing once per day Since apomorphine will be used multiple times during the day, it is desirable that the in vivo micronucleus test be conducted using a multiple dose per day regimen

The sponsor has requested a waiver from conducting carcinogenicity studies The sponsor's arguments are not persuasive The sponsor states that patients may be on apomorphine for up to six years ICH guideline S1A recommends that carcinogenicity studies be conducted on drugs likely to be used for two to three years Thus, the lack of carcinogenicity studies is a major data gap in the preclinical database

The sponsor has also requested a waiver from conducting reproductive toxicity studies The sponsor's arguments are not persuasive, especially since their own clinical studies included patients with reproductive potential There are also reports in the scientific literature of pregnant

patients with Parkinson's disease who experience worsening of off states, the indication that the sponsor intends to treat with this drug The sponsor's arguments are not sufficient to support the waiver of reproductive toxicity studies This is another major data gap in the preclinical database

The chemistry section of the application sets specification of — for a pair of degradation products in the drug product This is above the threshold for qualification — at the proposed clinical dose levels The sponsor needs to either lower the specification for these impurities or conduct a qualification study (a 13 week study in a single species) to assess the potential for the degradation product to affect the safety of the drug product This reviewer does not consider it necessary to conduct genotoxicity studies to qualify the degradation products since apomorphine is strongly genotoxic in its own right

In summary, preclinical studies suggest that the dose-limiting toxicity associated with apomorphine are central nervous system signs associated with excessive pharmacological stimulation of dopamine receptors The studies also suggest that there is potential for effects on the male reproductive system, but definitive reproductive toxicity studies have not conducted in male or female animals Carcinogenicity studies have not been conducted, but the sponsor has requested permission to conduct these studies post approval, which is acceptable This reviewer considers this application to be approvable pending completion of carcinogenicity studies, a reproductive toxicity battery and qualification studies for the degradation product (assuming that the sponsor is unable to lower the specification for the degradation product) Studies that can be conducted as phase IV commitments include the metabolism studies, the in vivo micronucleus test, and the combination study

PHARMACOLOGY

No pharmacology studies were submitted

APPEARS THIS WAY
ON ORIGINAL

SAFETY PHARMACOLOGY

No safety pharmacology studies were submitted

APPEARS THIS WAY
ON ORIGINAL

PHARMACOKINETICS/TOXICOKINETICS

PK parameters
No data submitted

Absorption
No data submitted

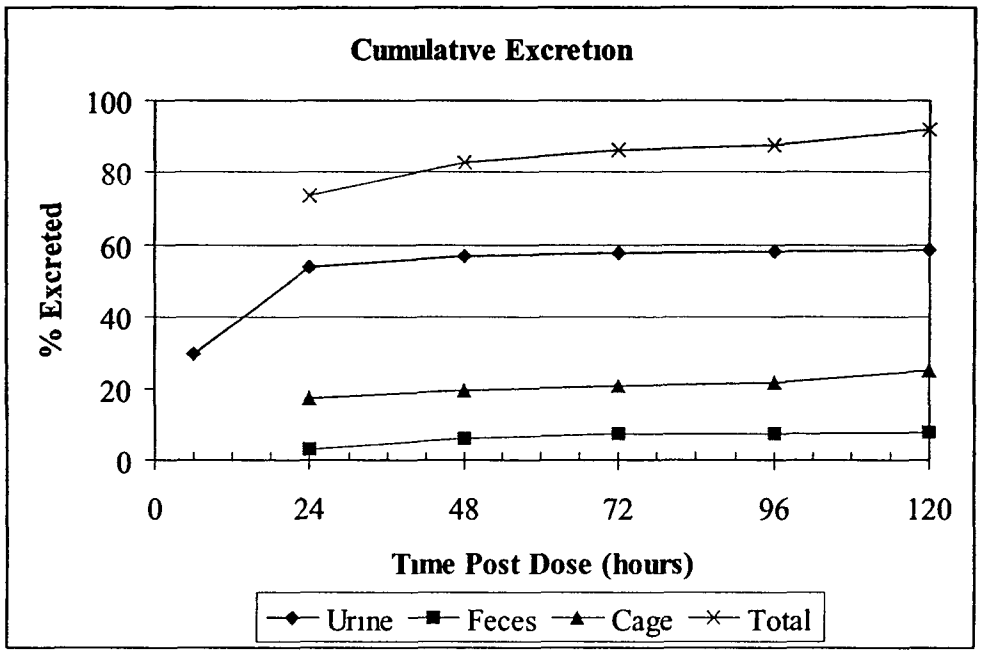
Distribution
No data submitted

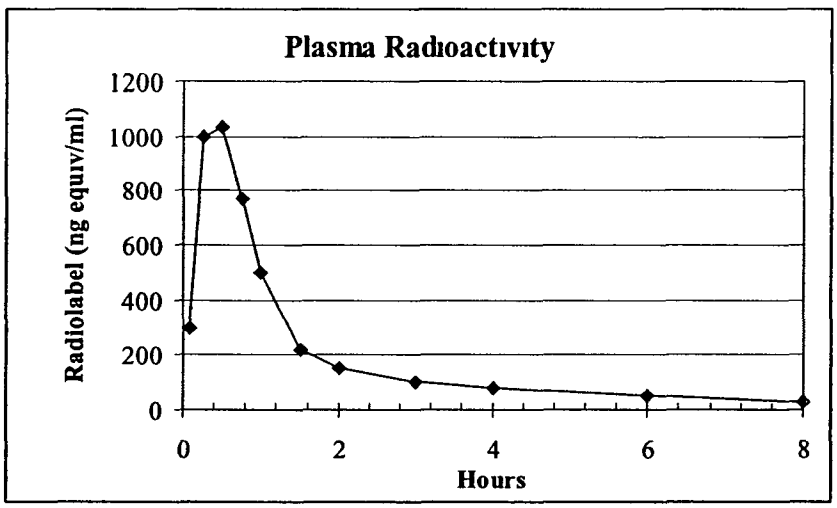
Metabolism

IN VITRO METABOLISM OF APOMORPHINE BY HUMAN LIVER MICROSOMES
Study No Report – 99059
Location Section 5 / Volume 3 / Page 1
Apomorphine was incubated with human microsomes. A single metabolite (presumed to be norapomorphine) was detected, although there was only minimal metabolism. Based on cDNA incubation with inhibitors, the primary cytochrome P450 isozymes involved in metabolism are CYP2B6, CYP2C8 and CYP3A4.

Excretion

THE METABOLISM AND PHARMACOKINETICS OF [14C]-APOMORPHINE IN THE CYNOMOLGUS MONKEY
Study No — Report No. 8962
Location Section 5 / Volume 1 / Page 299
Cynomolgus monkeys (2/sex) were administered 0.5 mg/kg subcutaneously. Urine and feces collected for up to 120 hours post dose. Blood samples collected at 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 24, 48, 72, 96 and 120 hours post dose.
Insufficient plasma radioactivity was available to analyze metabolites, virtually all of the radiolabel was cleared by 24 hours.
Urinary and fecal metabolites were not positively identified, but apomorphine was metabolized to a more polar compound and subsequently conjugated with glucoronic acid.





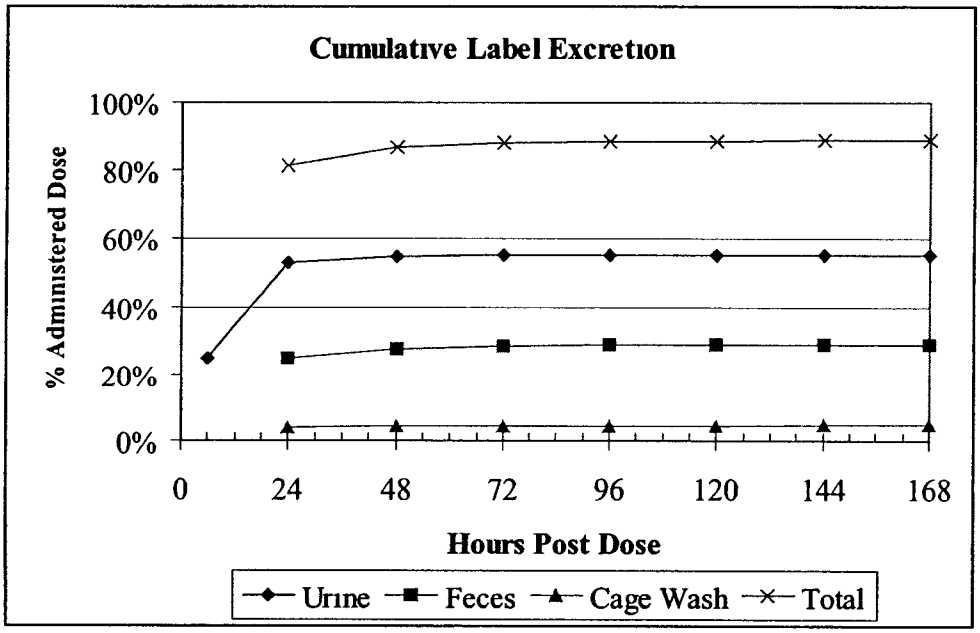
THE DISPOSITION OF [^{14}C]-APOMORPHINE IN THE RAT FOLLOWING SUBCUTANEOUS ADMINISTRATION

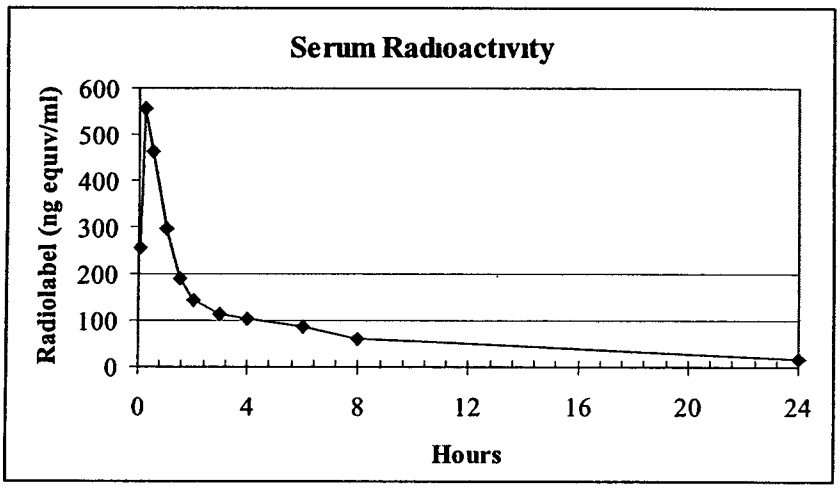
Study No — Report No 8963

Location Section 5 / Volume 1 / Page 203

Male Sprague-Dawley rats (n=5) were injected subcutaneously with 1 mg/kg [14C]-labelled apomorphine and excretion of label was monitored for 168 hours In the second phase of the study, male rats (n=5) were injected subcutaneously with 1 mg/kg [14C]-labelled apomorphine and blood was collected from the tail vein at 0 08, 0 25, 0 5, 1, 1 5, 2, 3, 4, 6, 8, 24, and 48 hours postdose

Most of the radioactivity was excreted by 24 hours post injection The urine was the primary mode of excretion (accounting for 60-65% of excreted label), the feces was also a major route of elimination (accounting for 30-32% of excreted label) Virtually no label was observed in expired air Apomorphine label was rapidly cleared from the blood About 70% of the radiolabel in the feces was associated with the parent apomorphine peak About 18% of the radiolabel in the urine was associated with the parent compound peak The major urinary apomorphine metabolite identified was the glucoronide conjugate (about 32% of label) Plasma metabolites were not analyzed





Other studies

IN VITRO EVALUATION OF APOMORPHINE HCL AS AN INDUCER OF CYTOCHROME P450 AND UDP-GLUCURONOSYLTRANSFERASE EXPRESSION IN HUMAN HEPATOCYTES

Study No Report — 99060-2
Location Section 5 / Volume 3 / Page 90

Apomorphine was incubated with human hepatocytes for three days The data suggest that apomorphine is a weak inducer of CYP1A2 (6 5-fold), CYP2B6 (2 1-fold), CYP2E1 (1 4-fold), and CYP3A4 (1 7-fold) The responses did not always follow a dose response relationship The significance of some of these findings is uncertain

EVALUATION OF APOMORPHINE HCL AS AN INHIBITOR OF CYTOCHROME P450 ENZYMES IN VITRO

Study No Report — 99058
Location Section 5 / Volume 3 / Page 151

Apomorphine was incubated with human hepatocytes and the effects on cytochrome P450 substrate metabolism was examined

Apomorphine was not a significant inhibitor of the tested P450 enzymes

Table 5 Summary of results Evaluation of Apomorphine HCl as an Inhibitor of human P450 enzymes

		Metabolism-independent inhibition				Metabolism-dependent inhibition	
Enzyme	P450 Activity	Ki (µmol/L)	[I]^{b}/Ki (µmol/L)	i	$i_{unbound}$ [d]	Reversible	Irreversible
CYP1A2	7 Ethoxyresorufin *O*-dealkylase	55 ± 12 [MI]	0 000645	0 0645%	0 000645%	Little or no inhibition	Little or no inhibition
CYP2C9	Diclofenac 4 -hydroxylase	370 ± 70 [CI]	0 000096	0 00959%	0 000096%	Little or no inhibition	Little or no inhibition
CYP2C19	*S* Mephenytoin 4 -hydroxylase	440 ± 170 [MI]	0 000081	0 00807%	0 000081%	Little or no inhibition	Little or no inhibition
CYP2D6	Dextromethorphan *O*-demethylase	50 ± 9 [MI]	0 000710	0 0710%	0 000710%	Little or no inhibition	Little or no inhibition
CYP2E1	Chlorzoxazone 6-hydroxylase	290 ± 40 [MI]	0 000122	0 0122%	0 000122%	Little or no inhibition	Weak inhibition
CYP3A4/5	Testosterone 6β-hydroxylase	33 ± 1 [NC]	0 001076	0 108%	0 001076%	Little or no inhibition	Little or no inhibition

Inhibition constants (Ki values) were calculated with the software program GraFit (version 4 0 Erithacus Software Ltd London U K) with simple weighting
Values are shown ± standard errors, which were calculated by GraFit using a matrix inversion method
[b] I = estimated plasma concentration of apomorphine in humans 0 0355 µM
i = predicted fractional inhibition *in vivo* based on the maximum human plasma concentration of 0 0355 µM
[d] $i_{unbound}$ = *predicted fractional inhibition in vivo based on greater than 99% protein binding*
MI Mixed inhibition CI Competitive inhibition NC Noncompetitive inhibition
Metabolism-dependent inhibition
Little or no inhibition 0 – 10% Inhibition Weak inhibition 11 – 20% Inhibition
Moderate inhibition 21 – 40% Inhibition Marked inhibition > 40% Inhibition

Figure 1, from page 30 of Report XT990058

TOXICOLOGY

Apomorphine 13 Week Toxicity Study in Rats with Subcutaneous Administration

Study no — **Project No 450855**
Volume #, and page # Section 5 / Volume 7 / Page 213
Section 5 / Volume 1 / Page 183 (TK report)
Conducting laboratory and location —
Scotland
Date of study initiation **February 1992**
GLP compliance **Yes**
QA report yes (X) no ( )
Drug, lot #, radiolabel, and % purity **200863**
Formulation/vehicle "control medium"

Methods (unique aspects)
Dosing

Species/strain	Rat, Sprague-Dawley
#/sex/group or time point (main study)	15/sex/dose
Satellite groups (toxicokinetics or recovery)	none
Age	5 weeks
Weight	about 144 g (males), 123 g (females)
Doses in administered units	0, 0 4, 1 0, 4 0 mg/kg/day
Route, form, volume, and infusion rate doses	SCU, 4 divided doses 1 5-2 hours between

Observations and times

Clinical signs	Several times per day
Body weights	1X/week
Food consumption	1X/week
Ophthalmoscopy	Pre, Week 11 (high dose and control only)
EKG	Not done
Hematology	Week 12/13
Clinical chemistry	Week 12/13
Urinalysis	Week 12/13
Gross pathology	Week 13
Organs weighed	Week 13
Histopathology	10/sex at 0 and 4 mg/kg, Full Society of Toxicologic Pathologist Battery, adrenals in 0 4 and 1 mg/kg males
Toxicokinetics	Days 1, 41, 90, pre, 10, 20, 30, 45 60 minutes post dose
Other	

Results

Mortality

0.4 mg/kg- 1 female due to trauma at blood collection

1.0 mg/kg- 1 males due to treatment (self mutilation)

4.0 mg/kg- 5 males, 1 female, two of these deaths (one male, one female) were humane sacrifices attributable to severe clinical reactions to treatment (self mutilation and/or hypermotility), one male death was due to poor clinical condition in week 11, the other three deaths were associated with exposure to a heating cabinet during pharmacokinetic sampling during week 13 of the study

Clinical signs No clinical signs in controls

Number of rats affect (number of observations)

	Males			Females		
	0.4 mg/kg	1.0 mg/kg	4 mg/kg	0.4 mg/kg	1.0 mg/kg	4 mg/kg
Ataxia		1/15 (1)	12/15 (15)			3/15 (4)
Slight		1 (1)	10 (10)			1 (1)
Moderate			5 (5)			2 (3)
Bite/Lick Cage	8/15 (8)	7/15 (13)	14/15 (114)	7/15 (7)	9/15 (25)	15/15 (149)
Slight	7 (7)	2 (2)	8 (12)	7 (7)	5 (5)	5 (9)
Moderate	1 (1)	6 (11)	14 (94)		9 (26)	15 (139)
Severe			3 (8)			1 (1)
Self Mutilation	0/15	7/15 (7)	5/15 (23)	0/15	2/15 (2)	7/15 (36)
Slight		1 (1)	1 (1)			
Moderate		3 (3)	5 (10)		1 (1)	6 (17)
Severe		3 (3)	2 (12)		1 (1)	5 (19)
Nervous	0/15	3/15 (3)	15/15 (48)	1/15	3/15 (5)	12/15 (51)
Slight			13 (24)	1 (1)	1 (2)	10 (34)
Moderate		2 (2)	12 (20)		2 (2)	8 (15)
Severe		1 (1)	2 (4)		1 (1)	2 (2)
Head Shaking	0/15	0/15	8/15 (12)	0/15	0/15	3/15 (4)
Subdued	0/15	1/15 (1)	10/15 (14)	0/15	0/15	4/15 (4)

Body weights Dose-dependent decrease in body weight in high dose (4 mg/kg/day) males (-14%), not much effect in females

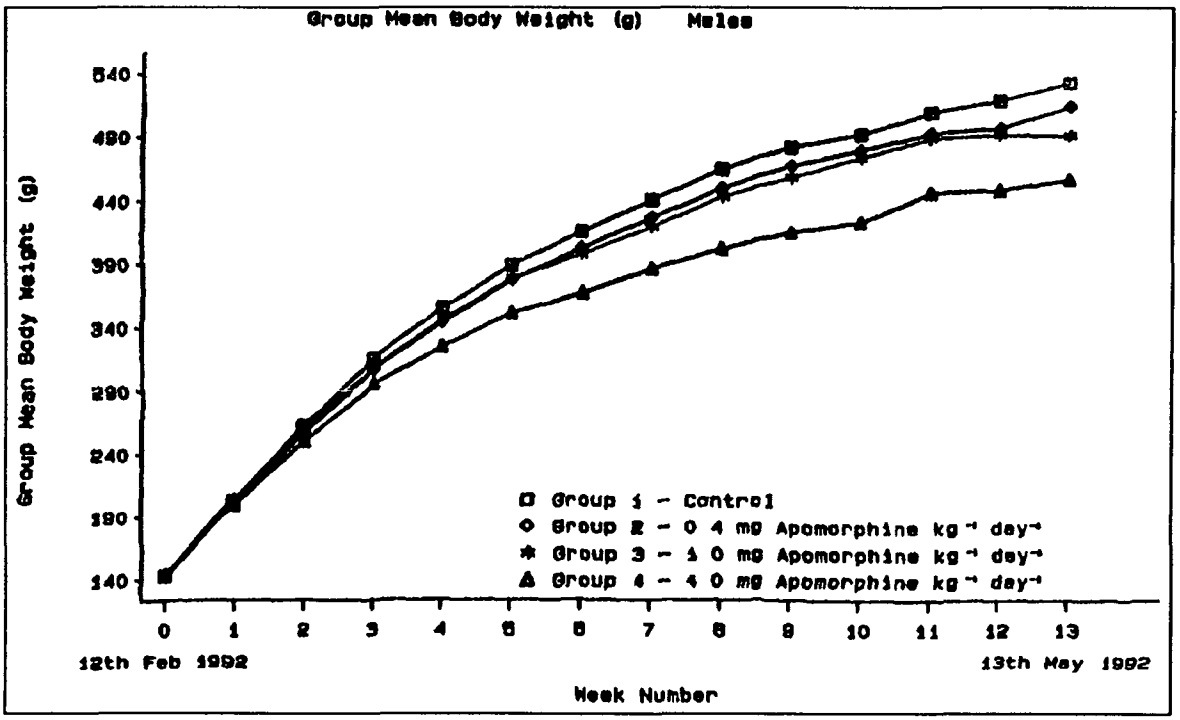


Figure 2, from page 67 of Report 450855

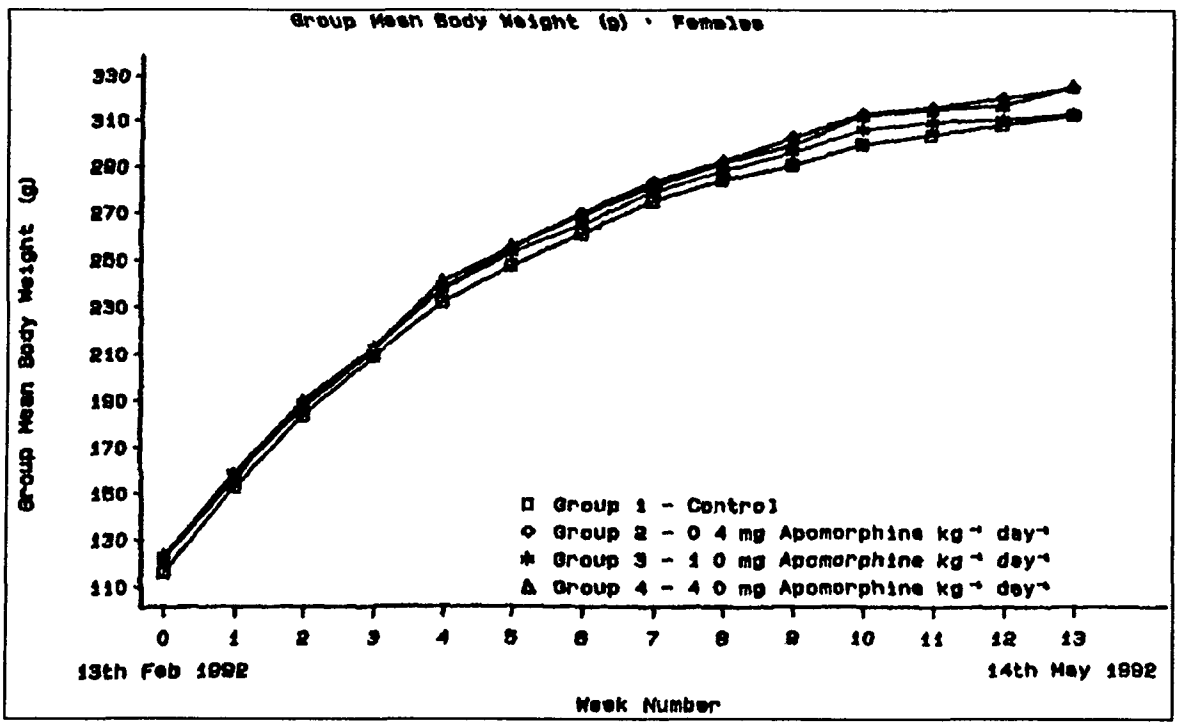


Figure 3 from page 68 of Project 450855

Food consumption decreased food consumption at 1 mg/kg (-5%) and 4 mg/kg (-7%) in males only

Ophthalmoscopy No effects

Electrocardiography: Not done

Hematology

Platelet concentrations ($x10^9$ / l)

Sex	0 mg/kg	0.4 mg/kg	1.0 mg/kg	4.0 mg/kg
Males	714	865	904	840
Females	703	731	**940**	**935**

Values in **Bold** significantly different from control

Clinical chemistry

Phosphorus concentrations (mmol / l)

Sex	0 mg/kg	0.4 mg/kg	1.0 mg/kg	4.0 mg/kg
Males	1.75	1.80	**1.94**	**1.96**
Females	1.70	1.80	**1.98**	**1.93**

Values in **Bold** significantly different from control

Urinalysis No effects

Organ weights

Mean organ weights transformed by covariance analysis in grams

Organ	Sex	0 mg/kg	0.4 mg/kg	1.0 mg/kg	4.0 mg/kg
Adrenals	Male	0.054	0.063	0.063	**0.066**
	Female	0.075	0.078	0.077	0.078
Kidney	Male	3.62	3.63	3.64	3.61
	Female	2.22	2.27	**2.37**	**2.41**
Ovary	Female	0.101	0.113	0.118	**0.142**
Thyroid	Male	0.034	0.029	0.026	**0.024**
	Female	0.025	0.023	0.022	0.021

Values in **Bold** significantly different from control

Gross pathology

	Sex	0 mg/kg	0.4 mg/kg	1.0 mg/kg	4.0 mg/kg
Injection Site, Dark	Male	9/15	9/15	9/15	7/15
	Female	4/15	2/15	4/15	6/15
Injection Site, Subcutaneous thickening	Male	0/15	0/15	0/15	1/15
	Female	0/15	0/15	0/15	1/15
Testes, Both small	Male	0/15	0/15	0/15	1/15

Histopathology

Incidence of Adrenal Cortical Hypertrophy in males (severity not assessed)

0 mg/kg	0 4 mg/kg	1 0 mg/kg	4 0 mg/kg
0/10	8/10	8/10	9/10

	Males		Females	
	0 mg/kg	4 0 mg/kg	0 mg/kg	4 0 mg/kg
Injection Site, subcutaneous reaction	7/7	6/6	2/2	4/4
Injection Site, focal myositis	1/7	1/6	0/2	0/4
Testes, Tubular Atrophy	0/10	1/10	---	---
Pancreas, focal interstitial lymphocytic infiltration	0/10	0/10	1/10	4/10

Toxicokinetics limit of quantitation was

Low concentrations (below limit of quantification) were observed at 0 1 mg/kg/dose (0 4 mg/kg/day) and 0 25 mg/kg/dose (1 0 mg/kg/day) There was no evidence of bioaccumulation Apomorphine was reported to have short half life (13-32 minutes) at 1 mg/kg/dose (4 mg/kg/day) This reviewer can not reproduce the half life's or AUC calculations The female 1 mg/kg/dose data was missing (Appendix F)

Key study findings

1 The primary adverse signs were clinical signs at 0 4 mg/kg/day and above Clinical signs included licking/biting the cage, self mutilation and subdued behavior The incidence and severity of the signs increased with increasing dose
2 Decreased body weight was observed at 4 0 mg/kg in males only
3 One high dose rat had testicular atrophy with decreased testes weight and small testes No other adverse effects on the testes in other rats
4 Adrenal cortical hypertrophy was observed in treated males, but not females

APPEARS THIS WAY
ON ORIGINAL

26-Week Subcutaneous Toxicity Study with Apomorphine in Rats with a 4-week Recovery Period

Study no — **9902**
Volume #, and page # Section 5 / Volume 11 / Page 1
Conducting laboratory and location. ——
Date of study initiation **August 16, 1999**
GLP compliance **Yes**
QA report yes (X) no ( )
Drug, lot #, radiolabel, and % purity **501413, — %**
Formulation/vehicle sodium metabisulfie 1 mg/ml in water for injection

Methods (unique aspects)
Dosing

Species/strain	Rat, Sprague-Dawley, — CD(SD)IGS BR
#/sex/group or time point (main study)	20/sex/dose
Satellite groups (recovery)	10/sex in control and high dose
Age	6 weeks
Weight	184-241 g (males), 138-184 g (females)
Doses in administered units	0, 0.3, 1.0, 3.0 mg/kg/day (4 divided doses)
Route, form, volume, and infusion rate between doses	SCU, 1 ml/kg, 4 divided doses 1.5-2 hours

Observations and times

Clinical signs	1/day from day 1-14, then 1/week (38-39 observations/rat)
Body weights	1X/week
Food consumption	1X/week
Ophthalmoscopy	Pre, Week 26, 30
EKG	Not done
Hematology	Weeks 4, 13, 26, 30
Clinical chemistry	Weeks 4, 13, 26, 30
Urinalysis	Weeks 4, 13, 26, 30
Gross pathology	Days 187-188
Organs weighed	
Histopathology	High dose and control groups, Full Society of Toxicologic Pathologist Battery, macroscopic lesions and lung, liver and kidneys from all doses, premature decedents
Toxicokinetics	Not done
Other	4 week recovery

Results

Mortality not drug related

0 mg/kg- 1 male during week 19 (B14546, suppurative pyelonephritis)

0 3 mg/kg- 1 female during week 4 (B14662, cause of death not determined)

3 0 mg/kg- 1 male during week 26 (B14606, sacrificed moribund with aspiration pneumonia)

Clinical signs

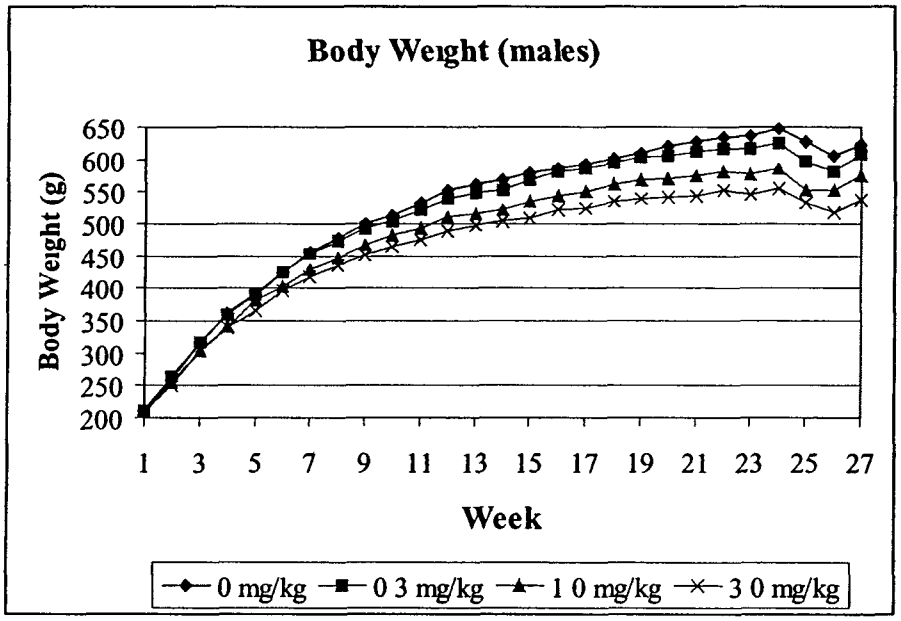
Incidence of Clinical Signs (percentage of observations with sign)-Males

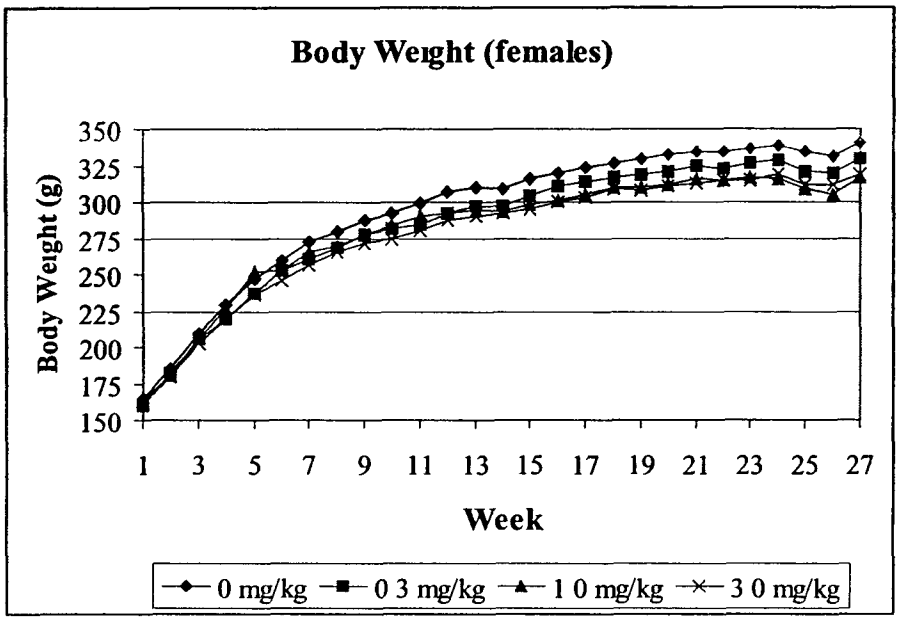
	0 mg/kg	0 3 mg/kg	1 0 mg/kg	3 0 mg/kg
Total observations	1141	770	770	1148
Excessive Sniffing	0/30 (0 0%)	6/20 (0 8%)	20/20 (90 9%)	30/30 (99 7%)
Excessive licking	0/30 (0 0%)	2/20 (0 3%)	18/20 (11 6%)	30/30 (37 9%)
Cage biting	0/30 (0 0%)	0/20 (0 0%)	10/20 (1 7%)	29/30 (17 1%)
Excessive grooming	0/30 (0 0%)	2/20 (0 4%)	0/20 (0 0%)	20/30 (7 1%)
Circling	0/30 (0 0%)	0/20 (0 0%)	0/20 (0 0%)	1/30 (0 1%)

Incidence of Clinical Signs (percentage of observations with sign)-Females

	0 mg/kg	0 3 mg/kg	1 0 mg/kg	3 0 mg/kg
Total observations	1150	747	770	1150
Excessive Sniffing	1/30 (0 1%)	10/20 (1 9%)	20/20 (90 9%)	30/30 (99 5%)
Excessive licking	0/30 (0 0%)	3/20 (0 4%)	18/20 (18 6%)	30/30 (39 4%)
Cage biting	4/30 (0 3%)	3/20 (0 5%)	15/20 (6 9%)	28/30 (25 7%)
Excessive grooming	0/30 (0 0%)	5/20 (1 1%)	5/20 (1 7%)	25/30 (8 6%)
Circling	0/30 (0 0%)	0/20 (0 0%)	0/20 (0 0%)	3/30 (0 3%)

Body weights Male body weights were decreased by 2, 8, and 14% at 0 3, 1 and 3 mg/kg, respectively, about an 8% decrease at 1 and 3 mg/kg in females





Food consumption	No effects
Ophthalmoscopy	No effects
Electrocardiography	Not done
Hematology	No effects
Clinical chemistry	

Tryglycerides in mg/dl

	Males				Females			
Week	0 mg/kg	0 3 mg/kg	1 mg/kg	3 mg/kg	0 mg/kg	0 3 mg/kg	1 mg/kg	3 mg/kg
4	82	66	59	62	46	48	49	43
13	119	74	**62**	62	61	53	63	50
27	113	89	**66**	**55**	108	**49**	50	**43**

Values in bold significant at $p<0.05$

Urinalysis	No effects
Organ weights	

Male Relative Organ Weights (%control)

	0 mg/kg	0 3 mg/kg	1 mg/kg	3 mg/kg
Kidney	0 567	0 599 (106%)	**0 617 (109%)**	**0 647 (114%)**
Heart	0 284	0 286 (101%)	**0 308 (108%)**	**0 326 (115%)**
Adrenal	0 0105	0 0108 (103%)	0 0116 (110%)	**0 0140 (133%)**

Lower testis weights were observed in rats with cryptorchid testes or hypospermia

Female Relative Organ Weights (%control)

	0 mg/kg	0 3 mg/kg	1 mg/kg	3 mg/kg
Heart	0 340	0 339 (100%)	0 353 (104%)	**0 364 (107%)**
Ovary	0 0382	0 0372 (97%)	0 0402 (105%)	**0 0518 (136%)**
Adrenal	0 0210	0 0238 (113%)	0 0253 (120%)	**0 0286 (136%)**

Gross pathology

3/19 high dose males had small testis (these three rats had cryptorchid testes)

Histopathology

5 high dose males had testicular abnormalities cryptorchid testis (3), hypospermia (1, also in epididymis), degeneration with mineralization (1), no abnormalities in 19 control males, low and mid dose groups were not examined

Injection Site

	Males		Females	
	0 mg/kg	3 mg/kg	0 mg/kg	3 mg/kg
Not remarkable	15/19	14/19	14/20	10/20
Hemorrhage	3/19	3/19	3/20	5/20
Inflammation, chronic	4/19	5/19	6/20	9/20
Ulcer	0/19	0/19	0/20	1/20
Acanthosis	0/19	0/19	0/20	1/20
Necrosis	0/19	0/19	0/20	1/20

Toxicokinetics Not done

Key study findings

1 A dose dependenct increase in the incidence of CNS signs indicative of stereotypic behavior (excessive grooming, cage biting and circling) was observed at all dose levels
2 Decreased body weights were observed at 1 mg/kg in males and 3 mg/kg in both males and females
3 5/19 3 mg/kg males has testicular abnormalities including small testes, cryptorchid testes (3), hypospermia (1) and degeneration (1) No changes in testicular parameters were observed in control rats
4 Increased body weight corrected ovary and heart weights were observed at 3 mg/kg/day

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A 13 Week Combination Toxicity Study of Combined Subcutaneous Apomorphine and Oral (Gavage) Levodopa/Carbidopa in Rats with a 4-week Recovery Period

Study no **3313 19 / Bertek Study No. Tox-018-002**
Volume #, and page # Section 5 / Volume 8 / Page 1
Conducting laboratory and location ———
Date of study initiation **April 24, 2001**
GLP compliance **Yes**
QA report yes (X) no ( )
Drug, lot #, radiolabel, and % purity **0L383**
Formulation/vehicle 1 mg/ml sodium metabisulfite aqueous solution

Methods (unique aspects)
Dosing

Species/strain	Rat, Sprague-Dawley — CD(SD)IGS BR
#/sex/group or time point (main study)	10/sex/dose
Satellite groups (recovery)	5/sex/dose (except sham control group)
Age	6-7 weeks
Weight	182-214 g (males), 175-208 g (females)
Doses in administered units	

Group	Male	Female	Targeted Dose Level Apomorphine (mg/kg/day)	Targeted Dose Level L-Dopa/Carbidopa (mg/kg/day)	Targeted Dose (mg/kg/dose) Apomorphine/ L Dopa + Carbidopa	Constant Dose Volume (mL/kg)
1 Sham Control	10	10	–	–	–	–
2 Vehicle Control	15	15	0	0/0	0 / 0 + 0	1
3	15	15	0	40/10	0/10 + 2 5	1
4	15 (17)	15 (17)	3	0/0	0 75 / 0 + 0	1
5	15 (17)	15 (17)	3	40/10	0 75 / 10 + 2 5	1
6	15	15	1	40/10	0.25 / 10 + 2 5	1
7	15	15	0 3	40/10	0 075 / 10 + 2 5	1

Note The last five animals/sex/group were maintained for a one-month recovery period. Animals designated with parentheses were utilized for a concurrent toxicokinetic phase

Figure 4, from page 19 of Study TOX-018-002

Route, form, volume, and infusion rate	Apomorphine-SCU injection 1 mgl/kg Levodopa/carbidopa- oral gavage

Observations and times

Clinical signs	2X/week
Body weights	1X/week
Food consumption	1X/week
Ophthalmoscopy	pre, Day 86
EKG	Not done
Hematology	Day 91/92
Clinical chemistry	Day 91/92
Urinalysis	Day 91/92
Gross pathology	Day 91/92
Organs weighed	
Histopathology	Control and high dose combination groups only (groups 1, 2, 5), Full Society of Toxicologic Pathologist Battery, lung, liver, kidney and gross lesions from low and mid-dose groups (3, 4, 6, 7)
Toxicokinetics	Days 5, 6, 7, groups 3 and 4 only

TK Sampling Schedule and Number of Male and Female Rats/Group at Sampling Time

Subgroup	Daily Dose 1		Daily Dose 2		Daily Dose 3		Daily Dose 4		
	0	15 min	0	15 min	0	15 min	0	15 min	90 min
Days 5 and 6									
A							4/4		
B								4/4	
Day 7									
C	3/3			3/3			3/3		
D		3/3			3/3			3/3	
E			3/3			3/3			3/3

Figure 5, from page 5 of Addendum to Toxicology Report TOX 018-002

Other

Results

Mortality

Group 1- female 942 sacrificed on day 14 after delivering pups

Group 3- female 962 sacrificed on day 45 due to mechanical injury in mouth area

Group 4- male 886 found dead on day 64 with heart thrombus

Clinical signs

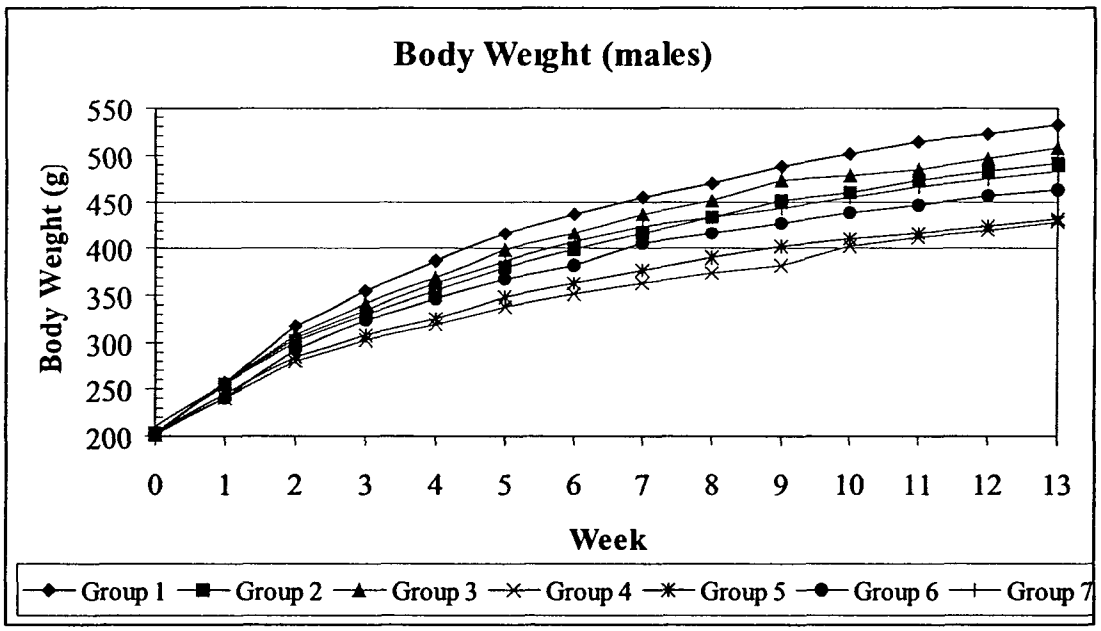
Males (occurances / animals affected)

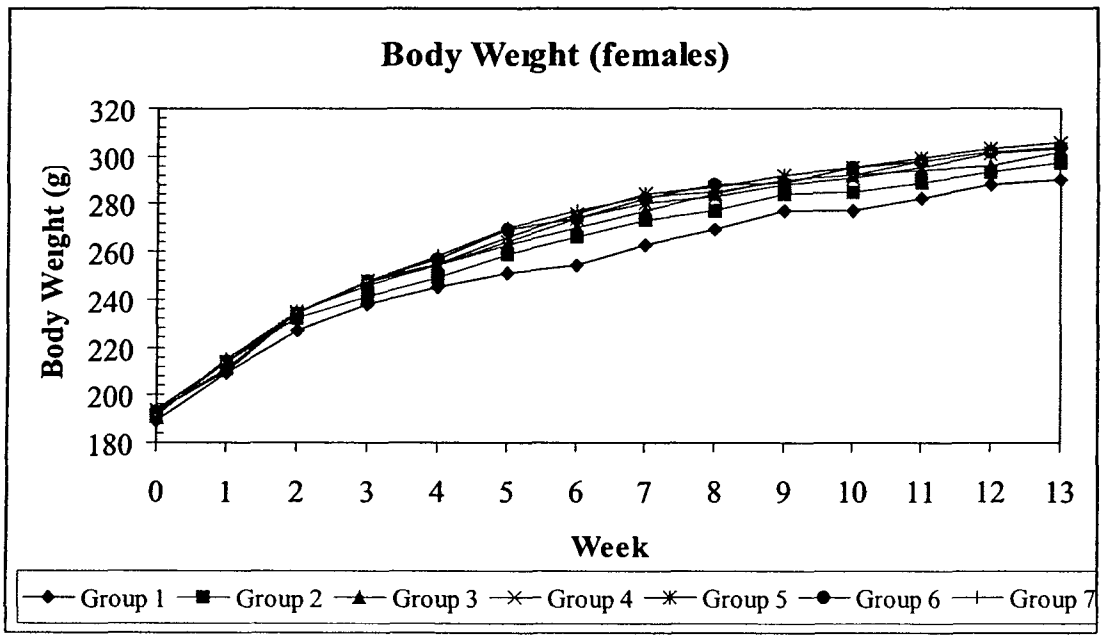
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Scabs	0/0	5/2	16/6	36/6	66/6	5/3	4/2
Unkempt	0/0	0/0	0/0	8/2	13/3	0/0	0/0
Vocalization	0/0	0/0	0/0	137/6	144/4	0/0	0/0
Increased activity post dose	0/0	0/0	0/0	494/13	404/11	0/0	0/0
Cage licking	0/0	0/0	0/0	337/15	316/15	317/15	4/4
Tail chewing	0/0	0/0	0/0	19/6	25/4	0/0	0/0
Circling	0/0	0/0	0/0	28/10	41/5	0/0	0/0

Females (occurances / animals affected)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Scabs	4/2	9/4	12/7	40/5	2/2	7/3	1/1
Increased activity post dose	0/0	0/0	0/0	18/6	8/6	0/0	0/0
Cage licking	0/0	1/1	0/0	345/15	370/15	365/15	1/1
Tail chewing	0/0	0/0	0/0	31/7	4/2	0/0	0/0
Circling	0/0	0/0	0/0	4/2	0/0	0/0	0/0

Body weights





Week 13 body weights as percent of Group 2 values

	Group 3	Group 4	Group 5	Group 6	Group 7
Males	95%	81%	81%	87%	91%
Females	104%	104%	106%	105%	105%

Food consumption	Decreased food consumption in groups 4 and 5
Ophthalmoscopy	No effects
Electrocardiography	Not done
Hematology	No effects
Clinical chemistry	No effects
Urinalysis	No effects
Organ weights	No effects
Gross pathology	

Injection Site-Males

	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Hemorrhage	5/10	3/10	4/9	6/10	8/10	7/10
Lesion	4/10	6/10	5/10	4/10	2/10	2/10

Injection Site-Females

	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Hemorrhage	9/10	7/9	8/10	9/10	8/10	9/10
Lesion	1/10	1/9	0/10	1/10	0/10	0/10

Histopathology

The injection sites were characterized has having generally mild fibrosis, hemorrhage and chronic inflammation The incidence is similar in intensity and frequency across control and dose groups

No other noteworthy findings were observed

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Toxicokinetics

Limit of qualification was – ng/ml

None of 12 samples taken prior to the first dose of the day had quantifiable apomorphine levels Three of the 60 samples taken 90 minutes after dosing had quantifiable apomorphine levels (— — ng/ml, samples taken after second dose of the day, third dose of the day and fourth dose of the day, respectively) Two observations were in the apomorphine alone (1 male and one female), one was in a female combination rat

Mean Apomorphine Plasma Concentration (ng/ml) at 15 Minutes Post Injection In Rats Treated With Apomorphine Alone (APO) and a Combination of Apomorphine and Sinemet (COMBO)

		Males		Females	
Day	Dose	APO	COMBO	APO	COMBO
5	4	99	95	97	92
6	4	94	81	86	117
7	1	79	80	80	79
	2	84	80	101	77
	3	88	79	70	92
	4	86	99	87	95

Key study findings

1 Apomorphine effects were similar to those in other studies in rats Decreased body weight and stereotypic behavior at 1 mg/kg and above
2 No signs of interaction effects, but the levodopa/carbidopa dose (50 mg/kg) were lower than generally used in these studies (150 mg/kg)
3 Apomorphine did not appear to accumulate with repeated exposures Between 15 minutes and 90 minutes, apomorphine would go through five half life's (assuming 15 minute half life) This implies that the 90 minute plasma concentration would be 1/32 the 15 minute plasma level It is not surprising that the 90 minute plasma concentrations were generally below the limit of quantification

Apomorphine 13 Week Subcutaneous (Bolus) Toxicity Study in Cynomolgus Monkeys

Study no — Project No 651585
Volume #, and page # Section 5 / Volume 13 / Page 59
Conducting laboratory and location ——
Tranent, Scotland
Date of study initiation **February 12, 1992**
GLP compliance **Yes**
QA report yes (X) no ( )
Drug, lot #, radiolabel, and % purity **200863**
Formulation/vehicle

Methods (unique aspects)
Dosing

Species/strain	Monkey, Cynomolgus (*Macaca fascicularis*)
#/sex/group or time point (main study)	4/sex/dose
Satellite groups (toxicokinetics or recovery)	None
Age	4 years
Weight	2 3-3 0 kg
Doses in administered units	0, 0 6, 1 5, 3 0 mg/kg/day
Route, form, volume, and infusion rate	6 divided SCU injections, 0 1 ml/kg over four regions

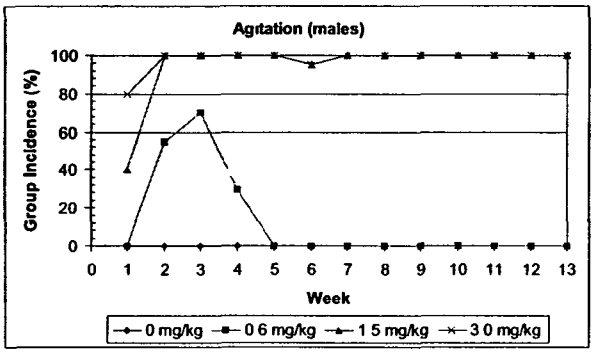
Observations and times

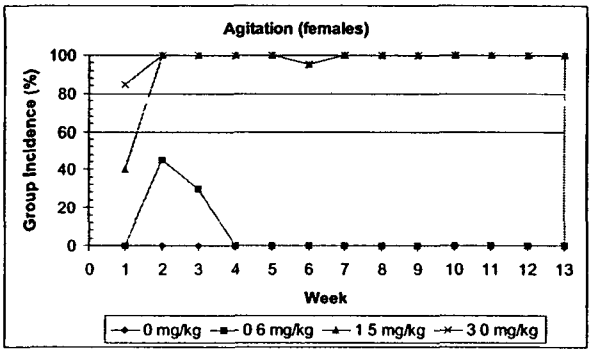
Clinical signs	"regular intervals, particularly after dosing, throughout the working day"
Body weights	1X/week
Food consumption	1X/day
Ophthalmoscopy	Pre, Weeks 6 and 13
EKG	Not done
Hematology	Pre, Weeks 6 and 13
Clinical chemistry	Pre, Weeks 6 and 13
Urinalysis	Pre, Weeks 6 and 13, feces examined for occult blood
Gross pathology	Week 13
Organs weighed	Week 13
Histopathology	All doses examined, Full Society of Toxicologic Pathologist Battery except epididymis, seminal vesicles, vagina, bone and bone marrow were not examined
Toxicokinetics	Day 1, Weeks 6 and 13, 0 (predose), 5, 10, 20, 30, 45 and 60 minutes post dose
Other	

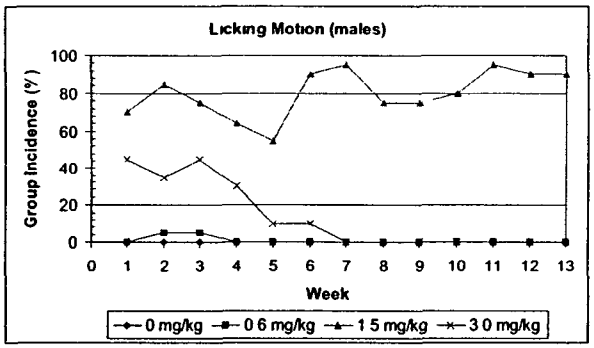
Results

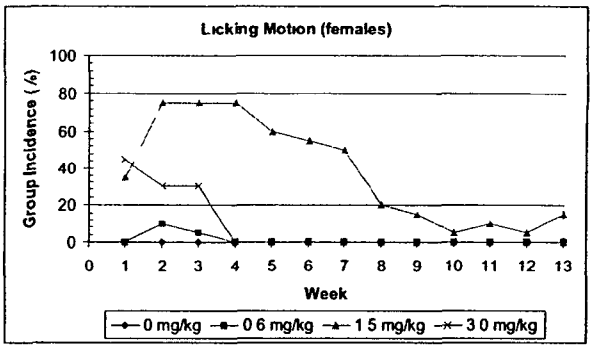
Mortality None

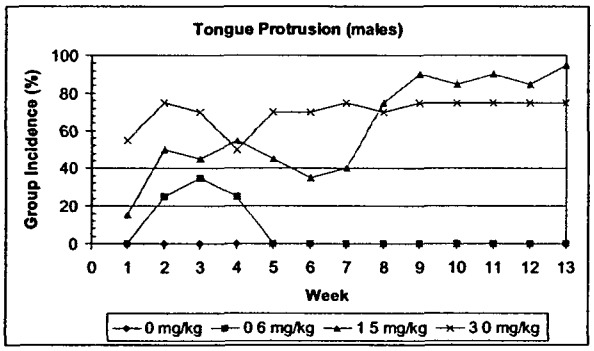
Clinical signs Greater severity of signs at higher doses, two monkeys (5 and 15) had their tails amputated, in part due to self mutilation Greater detail needed in other clinical signs

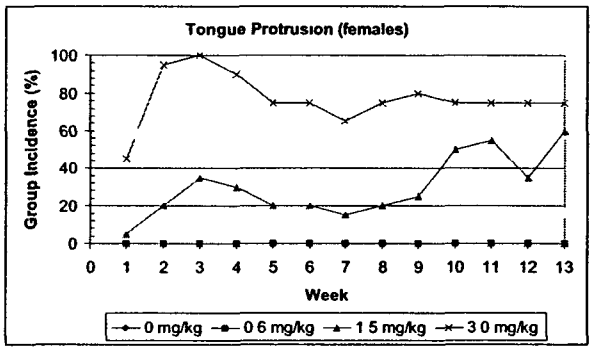




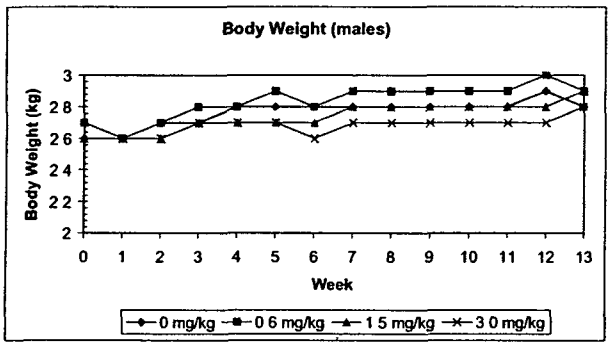


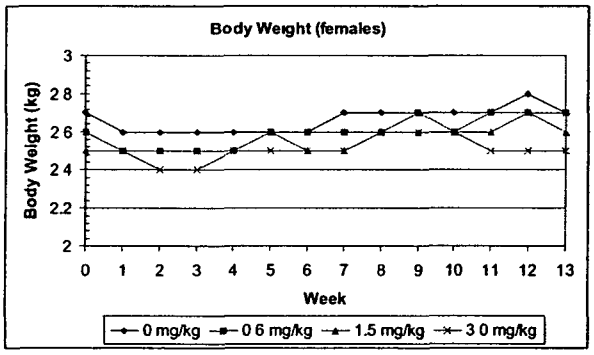






Body weights No effects





Food consumption No effect
Ophthalmoscopy No effects
Electrocardiography Not done
Hematology No effects
Clinical chemistry

Serum Phosphorous Concentrations in mmol/l

Sex	Males				Females			
Dose	0	0 6	1 5	3 0	0	0 6	1 5	3 0
Week 0	1 59	1 63	1 75	1 69	1 87	1 70	2 08	1 90
Week 6	1 81	1 63	1 68	**0 69**	1 69	1 78	1 56	1 12
Week 13	1 37	1 66	1 73	1 00	1 72	1 69	1 38	1 05

Serum Triglyceride Concentrations in mmol/l

Sex	Males				Females			
Dose	0	0 6	1 5	3 0	0	0 6	1 5	3 0
Week 0	0 41	0 38	0 47	0 51	0 64	0 46	0 50	0 80
Week 6	0 43	0 65	**0 95**	**1 00**	0 58	0 49	0 77	**1 09**
Week 13	0 80	0 87	0 74	0 79	0 64	0 56	0 73	0 96

Urinalysis No adverse effects
Organ weights

Organ Weights (covariance analysis)

Sex	Males				Females			
Dose	0	0 6	1 5	3 0	0	0 6	1 5	3 0
Adrenal	0 455	0 454	0 458	0 578	0 529	0 505	0 535	0 548
Liver	71 80	73 82	74 92	**91 12**	66 02	70 44	78 31	87 92
Kidney	13 75	15 18	15 39	**17 97**	14 68	12 64	13 84	16 43
Ovary	---	---	---	---	0 183	0 182	0 202	0 251
Thyroid	0 348	0 323	0 313	0 233	0 327	0 277	0 271	0 224
Thymus	3 64	4 82	3 38	2 42	3 01	2 32	3 82	1 94
Testes	1 91	1 69	1 68	1 75	---	---	---	---

Gross pathology

Incidence of subcutaneous thickening at the injection site

	0 mg/kg	0 6 mg/kg	1 5 mg/kg	3 0 mg/kg
Male	1/4	3/4	4/4	4/4
Female	1/4	4/4	4/4	4/4

Histopathology:

Two cases of thymic atrophy were observed in high dose monkeys (one female and one male), one case of focal thymic atrophy was observed in a mid dose female

Injection Site Histopathology

Sex	Males				Females			
Dose	0	0.6	1.5	3.0	0	0.6	1.5	3.0
Fibrous reaction	3/4	4/4	4/4	4/4	3/4	4/4	4/4	4/4
Minimal	1				2	1		1
Slight	1	2	1	2	1			2
Moderate	1	2	3	2		3	3	1
Severe							1	
Inflammation	4/4	4/4	4/4	4/4	3/4	4/4	4/4	4/4
Minimal					1			
Slight	3	1	1		2	1	1	2
Moderate	1	3	2	3		3	3	2
Severe			1	1				
Myositis	0/4	2/4	2/4	1/4	1/4	1/4	1/4	2/4

Toxicokinetics

Unspecified technical problems limited the data obtained. No TK data were obtained on several monkeys

0.6 mg/kg/day- Males: monkey 5 (no data), monkey 6 (no week 1 data), monkey 7 (missing single timepoint at weeks 1 and 13)

Females: no missing data

1.5 mg/kg/day-Males: monkey 9, (missing timepoint at week 13), monkey 12 (missing timepoint at week 6)

Females: monkey 25 (no data), monkey 26 (no data)

3 mg/kg/day-Males: monkey 13 (missing timepoints at weeks 6 and 13), monkey 14 (missing timepoint at week 1), monkey 15 (no data), monkey 16 (no data)

Females: no missing data

Males mean (range)

	Week	Cmax (nM)	$AUC_{(0-inf)}$ (nM-min)	Half life (min)
0.6 mg/kg	1	109 —	2,585 (2,450-2,720)	12.2 (12.1-12.3)
	6	99 —	2,275 (1,990-2,560)	14.4 (12.7-16.1)
	13	86 —	2,120 (1,770-2,680)	12.0 (10.9-12.6)
1.5 mg/kg	1	224 —	6,020 (4,890-6,960)	12.2 (9.8-14.0)
	6	188 —	5,078 (4,400-5,410)	15.8 (8.3-14.4)
	13	262 —	6,207 (5,140-7,970)	12.4 (9.0-15.1)
6.0 mg/kg	1	581 —	12,850 (10,800-14,900)	13.6 (13.4-13.7)
	6	428 —	12,600 (12,600)	11.4 (9.1-13.6)
	13	321 —	10,300 (10,300)	12.1 (11.3-12.8)

Females mean (range)

	Week	Cmax (nM)	$AUC_{(0\text{-}inf)}$ (nM-min)	Half life (min)
0 6 mg/kg	1	94 (—	2,115 (1,780-2,470)	13 5 (12 1-15 3)
	6	85 —	1,773 (1,530-2,100)	10 2 (9 7-11 1)
	13	92 —	1,873 (1 670-2,130)	14 4 (13 0-15 8)
1 5 mg/kg	1	290 —	5,785 (4,440-7,130)	11 3 (10 5-12 1)
	6	161 —	4,505 (4,490-4,520)	9 8 (8 2-11 3)
	13	234 —	5,425 (4,670-6,180)	10 8 (10 3-11 3)
6 0 mg/kg	1	552 —	13,500 (11,500-16,200)	12 0 (10 8-13 0)
	6	321 —	9,840 (8,150-11,800)	12 0 (10 5-13 2)
	13	335 —	10,638 (8,250-11,600)	11 0 (9 2-13 8)

Key study findings

1 CNS signs (agitation, tongue protrusion, licking motions) were observed starting at 0 6 mg/kg Self mutilation was observed at low and high dose males
2 No effects were observed on body weight
3 Some decreases in serum phosphorous and increased triglycerides were observed at 3 mg/kg
4 Increased severity of tissue reactions were observed in treated monkeys
5 Changes in organ weight were observed In the absence of histological changes, the significance of this observation is uncertain
6 Apomorphine was eliminated rapidly No potential for accumulation TK values proportional to dose

39-Week Subcutaneous Toxicity Study with Apomorphine in Cynomolgus Monkeys

Study no — 6482-117
Volume #, and page # Section 5 / Volume 14 / Page 1
Conducting laboratory and location —
Date of study initiation **August 11, 1999**
GLP compliance **Yes**
QA report yes (X) no ( )
Drug, lot #, radiolabel, and % purity **501413**
Formulation/vehicle sodium metabisulfite with sterile water for injection

Methods (unique aspects)
Dosing

Species/strain	Monkey, cynomolgus (*Macaca fascicularis*)
#/sex/group or time point (main study)	4/sex/dose
Satellite groups (recovery)	2/sex in high and control groups
Age	2-7 years
Weight	1 9-3 0 kg (males), 1 9-2 4 kg (females)
Doses in administered units mg/kg	0, 0 3, 1 0, high dose (see table below)

Dosing Interval	Group 4 *Total Dose Level* (mg/kg/day)	Group 4 *Dose Level* (mg/kg/dose)	Group 4 *Concentration* (mg/mL)	*Dose Volume* (mL/kg/dose)
Days 1 6	3 0	0.5	5 0	0 1
Days 7 14	0	0	0	0
Days 15 - 49	1.25	0.21	2.1	0 1
Days 50 - 70	1.5	0.25	2.5	0 1
Day 71	1 75	0.29	2 9	0 1
Day 72 termination	1 5	0.25	2.5	0 1

Figure 6, from page 14 of Report 6482-117

Route, form, volume, and infusion rate divided doses administered six times per day (1 5 hours in between injection) by SCU injection

Observations and times

Clinical signs	2X/day
Body weights	1X/week
Food consumption	
Ophthalmoscopy	Pre, Week 39
EKG	Pre, Week 39, prior to first dose of the day (approximately 16-17 hours after the last dose)
Hematology	Pre, Weeks 4, 13, 39
Clinical chemistry	Pre, Weeks 4, 13, 39
Urinalysis	Pre, Weeks 4, 13, 39
Gross pathology	Week 39
Organs weighed	

Histopathology Full Society of Toxicologic Pathologist Battery
Toxicokinetics Not done
Other

Results

Mortality One high dose monkey was sacrificed humanely on day 71 (dose was 1 75 mg/kg at the time), dehydration, thin appearance and hypothermia were noted

Clinical signs

Incidence of clinical observations in high dose males (number of weeks observation was made, %)

Dose	0 mg/kg	1 25 mg/kg	1 5 mg/kg	1 75 mg/kg	3 0 mg/kg
Weeks administered	2	3-7	8-40	11 (one day)	1
Total observations	6	30	168	6	6
Hyperactivity	3/6	6/6	6/6	5/6	6/6
Slight	3/6 (3, 50%)	6/6 (25, 83%)			
Moderate		4/6 (5, 17%)	6/6 (151, 90%)	2/6 (2, 33%)	
Severe			4/6 (17, 10%)	3/6 (3, 50%)	6/6 (6, 100%)
Aggressive	0/6	0/6	2/6 (3, 2%)	0/6	0/6
Eye, dilated	0/6	6/6 (9, 30%)	0/6	0/6	6/6 (6, 100%)

Incidence of clinical observations in high dose females (number of weeks observation was made)

Dose	0 mg/kg	1 25 mg/kg	1 5 mg/kg	1 75 mg/kg	3 0 mg/kg
Weeks administered	2	3-7	8-40	11 (one day)	1
Total observations	6	30	198	6	6
Hyperactivity	0/6	6/6	6/6	6/6	4/6
Slight		6/6 (25, 83%)	3/6 (5, 3%)		
Moderate		5/6 (5, 17%)	6/6 (176, 88%)	2/6 (1, 17%)	
Severe			6/6 (17, 9%)	5/6 (5, 83%)	4/6 (4, 67%)
Aggressive	0/6	0/6	3/6 (3, 2%)	0/6	0/6
Eye, dilated	0/6	4/6 (6, 20%)	0/6	0/6	3/6 (3/6, 50%)

Incidence of clinical signs in other dosed groups

	Males			Females		
	0 mg/kg	0 3 mg/kg	1 mg/kg	0 mg/kg	0 3 mg/kg	1 mg/kg
Total Observations	240	160	160	240	240	160
Hyperactivity	2/6	2/4	4/4	0/6	1/4	4/4
Slight	(2, 1%)	2/4 (9, 6%)	4/4 (108, 68%)		1/4 (2, 1%)	4/4 (24, 15%)
Moderate			4/4 (17, 16%)			
Severe			1/4 (1, 1%)			
Aggressive	0/6	0/4	0/4	0/6	0/4	0/4
Eye, dilated	0/6	1/4 (2, 1%)	4/4 (14, 9%)	0/6	0/4	4/4 (9, 6%)

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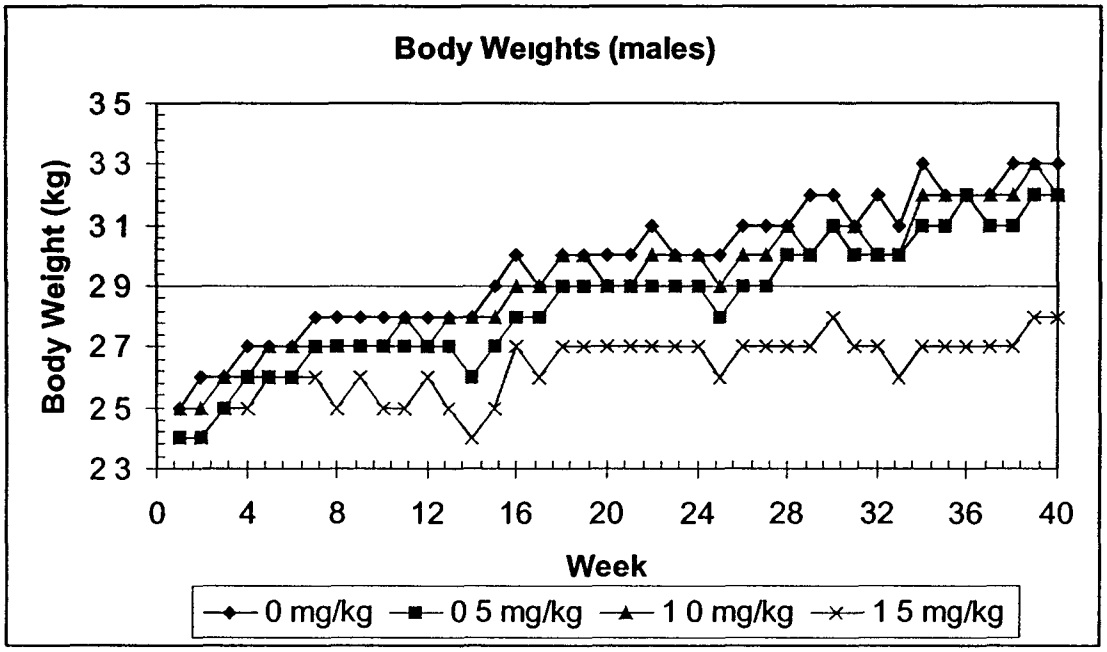
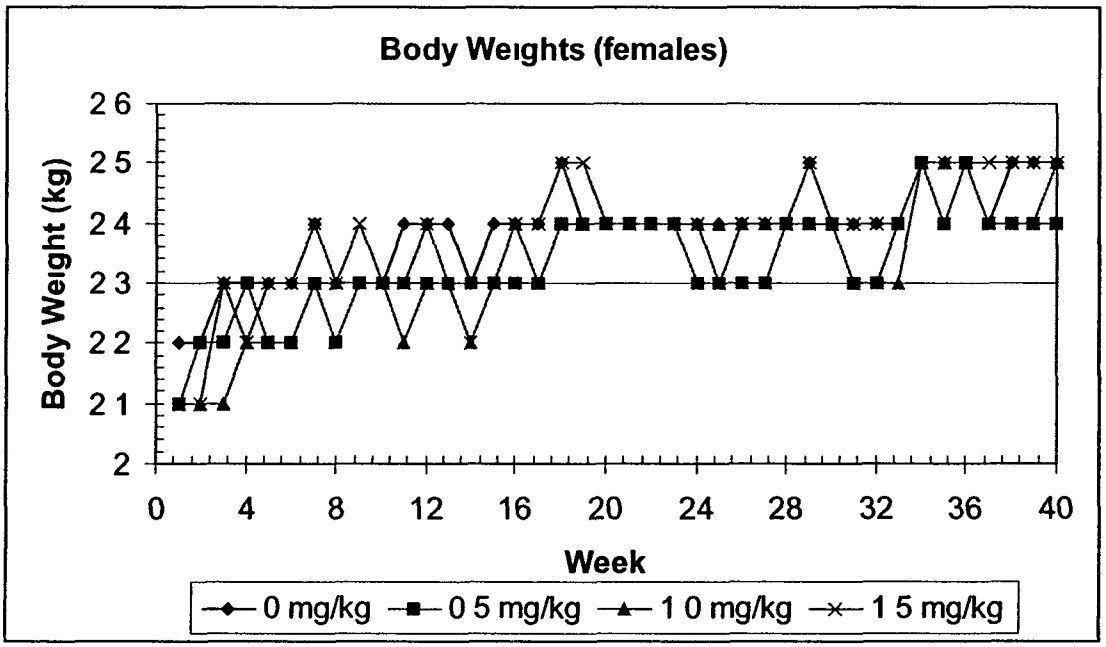
Body weights Decreased body weight (-15%) in high dose males only





Food consumption	no significant effects
Ophthalmoscopy	No effects
Electrocardiography	No adverse effects observed
Hematology	No effects
Clinical chemistry	No effects
Urinalysis	No effects

Organ weights

Relative Organ Weights (% of control)

	Males				Female			
	0	0 3	1 0	1 5	0	0 3	1 0	1 5
Liver	1 7	1 7 (100%)	1 8 (106%)	2 0 (118%)	2 0	2 1 (105%)	2 2 (110%)	2 2 (110%)
Testis	0 49	0 40 (82%)	0 26 (53%)	0 21 (43%)	---	---	---	---

Gross pathology

One high dose male monkey (50267) had kidney aplasia

Histopathology

Male

	0 mg/kg	0 3 mg/kg	1 0 mg/kg	1 5 mg/kg
Pancreas, Chronic inflammation	0/4	0/4	0/4	1/3
Testes, juvenile	1/4	0/4	1/4	1/3
Prostate, Chronic inflammation	1/4	1/4	3/4	3/3
Injection site, Chronic inflammation	3/4	4/4	3/4	3/3
Injection site, Muscle necrosis	1/4	0/4	0/4	1/3
Injection site, Hemorrhage	2/4	2/4	3/4	1/3

Female

	0 mg/kg	0 3 mg/kg	1 0 mg/kg	1 5 mg/kg
Brain, Foci of chronic inflammation	0/4	0/4	0/4	1/4
Stomach, Chronic inflammation	0/4	0/4	0/4	1/4
Pancreas, Chronic inflammation	0/4	0/4	1/4	1/4
Injection site, Chronic inflammation	4/4	3/4	4/4	4/4
Injection site, Muscle necrosis	0/4	0/4	0/4	0/4
Injection site, Hemorrhage	2/4	2/4	2/4	2/4

Toxicokinetics Not done

Key study findings

1 A dose-dependent increase in hyperactivity was noted in males starting at 0 3 mg/kg and in females starting at 1 0 mg/kg Dilated eyes were also observed at these doses, although this observation was primarily early in the study (first four weeks)
2 Decreased body weight was observed in males at 1 5 mg/kg
3 Decreased testis weight was observed Sponsor attributed decrease to juvenile testis, but only one juvenile testis was observed in the high dose group In addition, there was a dose-dependent decrease in testis weight across the doses Some decrease in testis weight was also observed in the 13 week monkey study
4 Increased liver weight was observed in treated monkeys This finding was also observed in the 13 week monkey study

5 Small increases in chronic inflammation were observed in various organs, but the severity was generally minimal to slight Significance is uncertain

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GENETIC TOXICOLOGY

Study to Determine the Ability of Apomorphine to Induce Mutation in Four Histidine Requiring Strains of Salmonella typhimurium and Two Tryptophan-Requiring Strains of Escherichia coli

Study no —— **27/S1**
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 15 / Page 54**
Conducting laboratory and location ——
Date of study initiation **October 8, 1991**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **103120, — % pure**
Formulation/vehicle **distilled water**

Methods

Strains/species/cell line: Salmonella typhimurium TA98, TA100, TA1535, TA1537, Eshcerichia coli WP2 pKM101, WP2 uvrA pKM101

Dose selection criteria

Basis of dose selection: cytotoxicity

Range finding studies: cytotoxicity at 1000 mg/plate

Test agent stability

Metabolic activation system: Aroclor 1254 induced male rat liver S9

Controls

Vehicle: Yes

Negative controls: solvent

Positive controls

Strain	-S9	+S9
TA98	2-Nitrofluorene	2-Aminoanthracene
TA100	Sodium Azide	2-Aminoanthracene
TA1535	Sodium Azide	---
TA1537	9-Aminoacridine	---
WP2 pKM101	4-Nitroquinoline-1-oxide	---
WP2 uvrA pKM101	4-Nitroquinoline-1-oxide	---

Comments

Exposure conditions

Incubation and sampling times: 72 hour incubation

Doses used in definitive study