Experiment	S-9	Concentration of treatment solution (mg/ml)	Final concentration (µg/ml)
Toxicity range-finder (0 5 ml additions of treatment solution for this experiment only)	- and +	0.016 0.080 0.40 2.00 10.00	8 40 200 1000 5000
Experiment 1 (all strains), and Experiment 2 (strains TA1535, TA1537 and WP2 pKM101)	-	0 25 0 50 1 00 2 00 4 00	25 50 100 200 400
Experiment 1 (all strains), and Experiment 2 (strains TA98, and TA1537)	+	0 50 1 00 2 00 4 00 8 00	50 100 200 400 800
Experiment 2 (strains TA98 and TA100)	-	0 125 0 250 0 500 1.00 2.00	12.5 25.0 50.0 100 200
Experiment 2 (WP2 uvrA pKM101 only)	-	0.0625 0 125 0.250 0.500 1.00	6.25 12.5 25 0 50.0 100
Experiment 2 (all strains except TA98 and TA1537)	+	0.50 1 00 2 00 4 00 6 00	50 100 200 400 600

Figure 7, from, page 11 of Report — 27/S1

Study design plate incorporation
Analysis
No of replicates triplicate plates, 5 for negative controls
Counting method electronic counting
Criteria for positive results positive Dunnett's test ($p \leq 0.01$)

Key findings·

Apomorphine was positive in TA98 and TA1537 in the presence and absence of metabolic activation

Experiment 1 in the absence of S9

	TA98	TA100	TA1535	TA1537	WP2 pKM101	WP2 uvrA pKM101
0	24	127	9	8	34	158
25	44	173	13	75	45	149
50	53	168	10	94	40	148
100	67	184	12	135	40	106
200	76 S	193 S	12	153	41	86 S
400	53 V	118 V	12 S	192 S	39 S	24 V
Positive	1657	606	527	845	134	1050

V=very thin background lawn

S=slight toxicity (thinning of background lawn or presence of microcolonies)

Experiment 1 in the presence of S9

	TA98	TA100	TA1535	TA1537	WP2 pKM101	WP2 uvrA pKM101
0	23	135	21	9	65	174
50	23	166	21	25	52	180
100	25	148	25	28	59	171
200	39	168	14	29	51	155
400	40	183	18	42	49	176
800	47	87 S	7 S	96 S	41 S	71 S
Positive	1299	1530	---	---	---	---

V=very thin background lawn

S=slight toxicity (thinning of background lawn or presence of microcolonies)

Experiment 2 in the absence of S9

	TA98	TA100	TA1535	TA1537	WP2 pKM101	WP2 uvrA pKM101
0	15	160	13	6	44	152
6 25	---	---	---	---	---	129
12 5	32	182	---	---	---	128
25	39	191	13	68	53	110
50	40	217	14	95	49	107
100	40	210	15	120	47	108
200	62	230	11	148	54	---
400	---	---	9 S	154 S,V	63 S	---
Positive	1669	470	464	979	204	1074

V=very thin background lawn

S=slight toxicity (thinning of background lawn or presence of microcolonies)

Experiment 2 in the presence of S9

	TA98	TA100	TA1535	TA1537	WP2 pKM101	WP2 uvrA pKM101
0	20	181	19	8	76	154
50	23	183	12	27	78	150
100	28	180	19	27	74	171
200	33	199	23	46	68	124
400	50	216	20	86	62	126
600	---	212 S	22	---	56	96
800	34	---	---	174	---	---
Positive	1098	1537	---	---	---	---

V=very thin background lawn

S=slight toxicity (thinning of background lawn or presence of microcolonies)

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Study to Determine the Ability of Apomorphine to Induce Mutation in Three Histidine Requiring Strains of Salmonella typhimurium

Study no —— 27/S2
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 15 / Page 117**
Conducting laboratory and location ——
Date of study initiation. **December 17, 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, TA1537
Dose selection criteria
- Basis of dose selection: previous study
- Range finding studies

Test agent stability
Metabolic activation system: Aroclor 1254 induced male rat liver S9
Controls
- Vehicle
- Negative controls
- Positive controls: 2-aminoanthracene (5 ug/plate) only used in the presence of metabolic activation
- Comments

Exposure conditions
- Incubation and sampling times: 72 hours
- Doses used in definitive study
- Study design: Plate incorporation, the mutagenicity of apomorphine was examined in the presence and absence of the antioxidants catalase, superoxide dismutase and glutathione

Analysis
- No. of replicates: triplicate plates, 5 for negative controls
- Counting method: electronic counting

Criteria for positive results: positive Dunnett's test ($p \leq 0.01$)

Key findings

Glutathione reduced the mutagenicity of apomorphine in the presence and absence of metabolic activation There was no control to examine the effect of glutathione on the mutagenicity of other test substances In addition, it appears that there were low negative control revertant colonies suggesting that glutathione may also affect the bacteria directly Superoxide dismutase had no effect on apomorphine mutagenicity Catalase increased the number of revertants in apomorphine free cultures, which makes it difficult to assess its effects on apomorphine mutagenicity

Strain	S-9	Apo dose		Catalase	SOD	GSH	+ Control
		ug/plate	0	5000 IU	250 IU	3 uMol	
TA98	-	0	26	52	25	18	
		150	72	70	72	23	
	+	0	38	55	44	29	1236
		600	98	106	85	37	
TA100	-	0	118	150	115	97	
		150	202	200	177	105	
	+	0	129	149	127	105	893
		400	225	229	234	135	

Results in TA1537

S-9	Apo Dose	Catalase (units/plate)				Positive Control
		0	2,500	5,000	10,000	
-	0	9	18	28	64	
	200	133	123	131	116	
+	0	7	9	27	41	52
	600	79	83	62	83	

Results in TA1537

S-9	Apo Dose	Superoxide Dismutase (units/plate)				Positive Control
		0	125	250	500	
-	0	9	9	7	6	
	200	133	142	139	142	
+	0	7	8	9	8	52
	600	79	99	77	100	

Results in TA1537

S-9	Apo Dose	Glutathione (umol/plate)				Positive Control
		0	1 5	3 0	4 5	
-	0	9	6	7	4	
	200	133	13	10	11	
+	0	7	7	10	7	52
	600	79	18	21	17	

Study to Determine the Ability of Apomorphine to Induce Mutation in Three Histidine Requiring Strains of Salmonella typhimurium

Study no. —— 27/S3
Study type (if not reflected in title)
Volume #, and page #: **Section 5 / Volume 15 / Page 148**
Conducting laboratory and location ——
Date of study initiation. **July 9, 1992**
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, TA1537
Dose selection criteria
Basis of dose selection: previous study
Range finding studies
Test agent stability
Metabolic activation system: Aroclor 1254 induced male rat liver S9
Controls
Vehicle
Negative controls
Positive controls

2-nitrofluorene (50 ug/plate) TA98 in the absence of metabolic activation
Sodium azide (2 ug/plate) TA100 in the absence of metabolic activation
9-aminoacridine (50 ug/plate) TA1537 in the absence of metabolic activation
2-aminoanthracene (5 ug/plate) all strains in the presence of metabolic activation

Comments
Exposure conditions
Incubation and sampling times: 72 hours
Doses used in definitive study
Study design: 20 minute apomorphine pre-incubation. Cells were then washed free of apomorphine and plated for 72 hours in the presence or absence of catalase (— units/plate), superoxide dismutase (— units/plate) or glutathione (— umol/plate). Positive controls used standard plate incorporation treatment
Analysis
No. of replicates: triplicate plates, 5 for negative controls
Counting method: electronic counting
Criteria for positive results: positive Dunnett's test ($p \leq 0.01$)

Key findings

In this assay system, apomorphine showed significant mutagenic activity in TA1537 (with or without metabolic activation) and borderline activity in TA98 (with metabolic activation) All other increases in mutation rate were less than two-fold Glutathione reduced the mutagenicity of apomorphine Since the cultures were washed free of apomorphine prior to treatment with glutathione, this suggests that glutathione does not directly interact with apomorphine, but rather enhances the repair of apomorphine induced damage

Strain	Apomorphine dose	No additive	Catalase	SOD	Glutathione	Positive
No Metabolic Activation						
TA98	0	26	41	31	21	1096
	93 75	40	46	33	22	
TA100	0	83	104	102	78	577
	93 75	132	128	105	96	
TA1537	0	8	35	10	7	523
	62 5	51	67	27	14	
With Metabolic Activation						
TA98	0	16	62	22	18	1164
	375	45	60	48	25	
TA100	0	86	114	92	79	1759
	375	148	140	119	85	
TA1537	0	13	44	12	13	320
	750	173	167	151	97	

Study to Determine the Ability of Apomorphine Hydrochloride to Induce Mutation in Five Histidine Requiring Strains of Salmonella typhimurium

Study no —— **Study No** **1/S**
Study type (if not reflected in title)
Volume #, and page #: **Section 5 / Volume 15 / Page 198**
Conducting laboratory and location: ——
Date of study initiation **February 20, 1991**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **19190, —— % pure**
Formulation/vehicle **distilled water**

Methods

Strains/species/cell line Salmonella typhimurium TA98, TA100, TA 102, TA1535, TA1537
Dose selection criteria
Basis of dose selection Range Finding Cytotoxicity
Range finding studies Up to 5000 ug/plate
Test agent stability
Metabolic activation system Aroclor 1254 induced male rat liver S9
Controls
Vehicle Distilled Water
Negative controls Vehicle
Positive controls

Strain	-S9	+S9
TA98	2-Nitrofluorene	2-Aminoanthracene
TA100	Sodium Azide	2-Aminoanthracene
TA102	Glutaraldehyde	---
TA1535	Sodium Azide	2-Aminoanthracene
TA1537	9-Aminoacridine	---

Comments
Exposure conditions
Incubation and sampling times 72 hours
Doses used in definitive study see below

Study design plate incorporation
Analysis
No of replicates triplicate plates (5 in negative controls)
Counting method electronic counting ——
Criteria for positive results Dunnett's test ($p<0.01$)

Key findings

Apomorphine was positive in TA98 and TA1537 in the presence and absence of S9. A dose-dependent increase in revertants TA100 was interpreted as a mutagenic effect, even though a doubling of revertants was not observed.

Experiment 1 in the absence of S9

	TA98	TA102	TA1535	TA1537
0	26	301	13	7
0.8	31	279	11	6
4	26	292	15	17
20	44	279	9	49
100	66	192	12	112
500	75 S	83 A T	9 S	114 S
Positive	1972	653	1173	715

A=absence of background lawn
S=slight toxicity (thinning of background lawn or presence of microcolonies)
T=toxic, no revertant colonies were observed in some plates at this dose

Experiment 1 in the presence of S9

	TA98	TA102	TA1535	TA1537
0	25	278	22	9
1.2	29	258	23	7
6	30	271	13	12
30	27	246	22	19
150	45	277	21	41
750	75	172	15	133
Positive	1248	---	151	---

Experiment 1 results with TA100

	0	0.32	1.6	8	40	200	1000	5000	Positive
-S9	130	140	143	137	155	219	0 T	0 T	1152
+S9	147	---	153	183	173	202	41 V	0 T	1048

V=very thin background lawn
T=toxic, no revertant colonies were observed in some plates at this dose

Experiment 2 in the absence of S9

	TA98	TA100	TA102	TA1535	TA1537
0	23	119	312	17	7
25	58	149	291	16	64
50	70	170	290	16	91
100	80	189	262	18	120
200	98	229	185	13	127
400	99 S	218 S	149 S	14	175 S
Postive	2027	1077	501	1057	626

S=slight toxicity (thinning of background lawn or presence of microcolonies)

Experiment 2 in the presence of S9

	TA98	TA100	TA102	TA1535	TA1537
0	33	140	376	24	6
50	30	150	407	23	21
100	38	158	389	24	26
200	48	161	403	21	55
400	71	173	352	24	87
800	83	214	263	13	192
Positive	1350	1233	---	---	---

Study to Determine the Ability of Apomorphine Hydrochloride to Induce Mutations at the Thymidine Kinase (tk) Locus in Mouse Lymphoma L5178Y Cells Using a Fluctuation Assay

Study no — **Study No** **1/TK**
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 15 / Page 244**
Conducting laboratory and location —
Date of study initiation **June 18, 1991**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **19190, — % pure**
Formulation/vehicle **distilled water**

Methods

Strains/species/cell line L5178Y mouse lymphoma cells
Dose selection criteria
Basis of dose selection Cytotoxicity in range finding study
Range finding studies
Test agent stability
Metabolic activation system aroclor 1254 induced male rat liver S9
Controls
Vehicle Yes
Negative controls
Positive controls 4-nitroquinoline-1-oxide (-S9), benzo(a)pyrene (+S9)
Comments
Exposure conditions
Incubation and sampling times 3 hours, 48 hour expression
Doses used in definitive study
Study design fluctuation protocol
Analysis
No of replicates duplicate
Counting method colonies identified by eye
Criteria for positive results reproducible statistically increase in mutant frequency with dose response relationship

Key findings.
Apomorphine reproducibly induced mutation in the presence and absence of metabolic activation The most significant increase was in frequency of small colonies suggesting a clastogenic effect

Experiment 1 without metabolic activation

Dose	Relative Survival	Mutation Frequency	Small Colonies	Large Colonies	Proportion Small
0	100	154	73	86	0 46
0 3125	97 9	156	---	---	---
0 625	92 5	142	---	---	---
1 25	92 5	209	---	---	---
2 5	92 5	214	---	---	---
5	83 6	275	161	108	0 60
10	27 7	643	424	210	0 67
Positive control 1	47 2	1000	529	229	0 70
Positive control 2	31 0	2000	1268	398	0 76

Experiment 1 with metabolic activation

Dose	Relative Survival	Mutation Frequency	Small Colonies	Large Colonies	Proportion Small
0	100	253	113	129	0 47
1 25	89 0	198	---	---	---
2 5	85 8	243	---	---	---
5	93 7	219	---	---	---
10	67 4	243	---	---	---
20	23 0	392	---	---	---
30	10 1	595	478	195	0 78
Positive control 1	61 6	877	619	175	0 78
Positive control 2	46 8	2000	1469	175	0 89

Experiment 2 without metabolic activation

Dose	Relative Survival	Mutation Frequency	Small Colonies	Large Colonies	Proportion Small
0	100	124	69	49	0 58
1 25	103 6	129	---	---	---
2 5	86 8	168	---	---	---
5	48 1	350	234	86	0 73
10	9 0	764	469	238	0 66
15	1 0	513	302	208	0 59
Positive control 1	59 5	772	492	158	0 76
Positive control 2	32 0	984	656	167	0 80

Experiment 2 with metabolic activation

Dose	Relative Survival	Mutation Frequency	Small Colonies	Large Colonies	Proportion Small
0	100	117	63	48	0 57
2 5	116 1	141	---	---	---
5	91	155	---	---	---
10	77 1	211	133	64	0 68
20	8 4	481	343	122	0 74
30	2 5	476	369	105	0 78
Positive control 1	88 4	660	357	226	0 61
Positive control 2	59 5	764	503	130	0 79

Study to Evaluate the Chromosome Damaging Potential of Apomorphine (Plus Reference Chemicals Epinephrine and Levodopa) by Their Effects on Cultured Human Lymphocytes Using an In Vitro Cytogenetics Assay

Study no — **Study No** 27, —
Study type (if not reflected in title):
Volume #, and page # **Section 5 / Volume 15 / Page 284**
Conducting laboratory and location —
Date of study initiation: **October 23, 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: freshly isolated lymphocytes from a single male donor
Dose selection criteria
- Basis of dose selection: Cytotoxicity
- Range finding studies: Yes

Test agent stability
Metabolic activation system: Aroclor 1254 induced male rat liver S9
Controls
- Vehicle
- Negative controls
- Positive controls: 4-Nitroquinoline-1-oxide (-S9), cyclophosphamide (+S9)
- Comments

Exposure conditions
- Incubation and sampling times: -S9 continuous for 20 or 44 hours, also 3 hours followed by 17 hour recovery period, +S9 3 hours followed by 17 or 41 hours recovery period
- Doses used in definitive study
- Study design

Analysis
- No. of replicates: duplicate (quadruplicate in negative control)
- Counting method

Criteria for positive results: statistically significant increases in the proportion of structurally aberrant cells (without gaps) occurred at one or more concentrations and the proportion of aberrant cells at such data points exceeded the normal range

Key findings

Apomorphine induced a pattern of genotoxicity in the presence and absence of metabolic activation in cultured human lymphocytes

20 Hour Expression without S9

Dose	Mitotic Index			Cells counted	%Cell Aberrations		Aberration Type (cells with aberration)
	A	B	Mean		-Gaps	+Gaps	
Control			9 3	200	0 0%	0 0%	---
56 5			6 3	200	2 5%	4 0%	Chromosome deletion (2) Chromatid deletion (3)
80 71			3 5	200	5 0%	7 5%	Chromosome deletion (1) Chromatid deletion (9)
115 3			2 9	200	5 5%	10%	Chromosome deletion (2) Chromatid deletion (9)
NQO				100	20 0%	25 0%	Chromosome deletion (1) Chromatid deletion (22) Chromatid exchanges (9) Other (3)

Other includes pulverized cells and cells with 7 or more aberrations

3 Hour Expression, 17 Hour Recovery with S9- Experiment A

Dose	Mitotic Index			Cells counted	%Cell Aberrations		Aberration Type (cells with aberration)
	A	B	Mean		-Gaps	+Gaps	
Control			4 8	200	0 0%	0 0%	---
235 3			3 8	200	4 0%	5 0%	Chromosome deletion (3) Chromatid deletion (5)
336 1			2 9	188	6 4%	9 0%	Chromosome deletion (6) Chromatid deletion (7) Chromatid exchange (2)
480 2			2 3	200	8 5%	4 0%	Chromosome deletion (3) Chromatid deletion (4) Chromatid exchange (2)
Cyclophosphamide				50	22 0%	28%	Chromosome deletion (1) Chromatid deletion (12) Chromatid exchange (1)

44 Hour Expression without S9

Dose	Mitotic Index			Cells counted	%Cell Aberrations		Aberration Type (cells with aberration)
	A	B	Mean		-Gaps	+Gaps	
Control			2 2	200	0 0%	0 0%	---
115 3			2 7	200	6 5%	8 0%	Chromosome deletion (10) Chromatid deletion (5)

3 Hour Expression, 41 hour recovery with S9

Dose	Mitotic Index			Cells	%Cell Aberrations		Aberration Type
	A	B	Mean	counted	-Gaps	+Gaps	(cells with aberration)
Control			7 6	200	1 0%	1 5%	Chromatid deletion (2)
480 2			3 8	200	2 5%	4 0%	Chromosome deletion (3) Chromatid deletion (3)

3 Hour Expression, 17 hour recovery without S9

Dose	Mitotic Index			Cells	%Cell Aberrations		Aberration Type
	A	B	Mean	counted	-Gaps	+Gaps	(cells with aberration)
Control			8 1	200	0 0%	0 5%	---
686			3 0	200	11 0%	14 0%	Chromosome deletion (4) Chromatid deletion (19)

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Study to Evaluate the Chromosome Damaging Potential of Apomorphine by Its Effects on Cultured Human Lymphocytes in the Absence and Presence of Superoxide Dismutase, Catalase and Glutathione

Study no — **Study No** 27/ —
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 16 / Page 16**
Conducting laboratory and location —
Date of study initiation **January 30, 1992**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **103120, — % pure**
Formulation/vehicle **distilled water**

Methods

Strains/species/cell line lymphocytes from a single male donor
Dose selection criteria
Basis of dose selection previous study
Range finding studies
Test agent stability
Metabolic activation system None
Controls
Vehicle yes
Negative controls distilled water
Positive controls none
Comments
Exposure conditions
Incubation and sampling times 44 hours
Doses used in definitive study
Apomorphine (APO)- 182.7 ug/ml
Superoxide Dismutase (SOD)- 275 units/ml
Catalase (CAT)- 80 units/ml
Glutathione (GSH)- 200 ug/ml
Study design cells incubated 44 hours
Analysis
No. of replicates duplicate
Counting method
Criteria for positive results statistically significant increase above historical control

Key findings

Superoxide dismutase appeared to lower the incidence of chromosomal aberrations in cultured lymphocytes treated with apomorphine On the other hand, glutathione greatly enhanced the incidence of chromosomal aberrations

44 Hour Expression without S9

Dose	Mitotic Index			Cells counted	%Cell Aberrations		Aberration Type (cells with aberration)
	A	B	Mean		-Gaps	+Gaps	
Control			4 8	200	1 0%	2 5%	Chromosome deletion (1) Chromatid deletion (2)
APO			2 7	300	7 3%	12 0%	Chromosome deletion (7) Chromatid deletion (18)
APO + SOD			2 7	200	1 0%	4 5%	Chromosome deletion (1) Chromatid deletion (1)
SOD			5 3	200	1 5%	2 5%	Chromosome deletion (1) Chromatid deletion (2)
APO + CAT			2 5	179	6 7%	10 6%	Chromosome deletion (6) Chromatid deletion (6)
CAT			5 1	200	2 5%	5 0%	Chromosome deletion (2) Chromatid deletion (3)
APO + GSH			1 7	150	31 2%	38 0%	Chromosome deletion (33) Chromatid deletion (46) Chromatid exchange (1)
GSH			5 6	200	1 5%	2 0%	Chromosome deletion (1) Chromatid deletion (2)

Numerical Aberrations

	Cells counted	Endoreduplication	Hyperdiploid	Polyploid	Total
Control	202	0	1	2	3 (1 5%)
APO	310	1	0	9	10 (3 2%)
APO + SOD	204	1	1	2	4 (2 0%)
SOD	202	0	1	1	2 (1 0%)
APO + CAT	187	1	2	5	8 (4 3%)
CAT	202	0	1	1	2 (1 0%)
APO + GSH	157	2	0	5	7 (4 5%)
GSH	200	0	0	0	0 (0%)

Study to Investigate the Clastogenic Potential of Apomorphine by Comparing Its Effects on the Chromosomes of Cultured Human Peripheral Blood Lymphocytes in the Absence and Presence of Glutathione

Study no — **Study No** 27—
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 16 / Page 37**
Conducting laboratory and location —
Date of study initiation **July 13, 1992**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **103120, —% pure**
Formulation/vehicle **distilled water**

Methods

Strains/species/cell line peripheral lymphocytes from single male donor
Dose selection criteria
Basis of dose selection previous study
Range finding studies
Test agent stability
Metabolic activation system Aroclor 1254 induced male rat liver S9
Controls
Vehicle water
Negative controls
Positive controls none
Comments
Exposure conditions
Incubation and sampling times 3 hour treatment, 17 hour recovery
Doses used in definitive study
Apomorphine (APO)- 590 6 ug/ml (-S9), 1050 ug/ml (+S9)
Glutathione (GSH)- 200 ug/ml (-,+S9)
Study design
Analysis
No of replicates duplicate
Counting method
Criteria for positive results

Key findings

Glutathione had essentially no effect on the genotoxic action of apomorphine in the presence of S9 The decrease in mutagenicity observed in the absence of S9 should be interpreted with care since there was a greater than 90% decrease in mitotic index

3 Hour Exposure, 17 hour Recovery Without S9

Dose	Mitotic Index			Cells	%Cell Aberrations		Aberration Type
	A	B	Mean	counted	-Gaps	+Gaps	(cells with aberration)
Control	—		5 0	200	1 0%	2 5%	Chromosome deletion (1) Chromatid deletion (1)
APO	—		1 1	139	13 7%	15 8%	Chromosome deletion (8) Chromatid deletion (15) Other (2)
APO + GSH	—		0 4	102	7 8%	12 7%	Chromosome deletion (4) Chromatid deletion (5) Other (1)
GSH	—		6 2	200	1 0%	2 0%	Chromosome deletion (2)

Other includes pulverized cells and cells with 7 or more aberrations

3 Hour Exposure, 17 hour Recovery With S9

Dose	Mitotic Index			Cells	%Cell Aberrations		Aberration Type
	A	B	Mean	counted	-Gaps	+Gaps	(cells with aberration)
Control	—		4 3	200	0 0%	0 5%	---
APO	—		0 6	169	13 0%	15 7%	Chromosome deletion (18) Chromatid deletion (1) Chromatid exchange (1) Other (2)
APO + GSH	—		1 8	200	16 5%	21 0%	Chromosome deletion (14) Chromatid deletion (19) Chromatid exchange (3) Other (2)
GSH	—		5 0	200	0 5%	0 5%	Chromatid deletion (1)

Other includes pulverized cells and cells with 7 or more aberrations

Numerical Aberrations 3 hour exposure in the absence of S9

	Cells counted	Endoreduplication	Hyperdiploid	Polyploid	Total
Control	201	1	0	0	1 (0 5%)
APO	142	0	0	3	3 (2 1%)
APO + GSH	105	1	0	2	3 (2 9%)
GSH	200	0	0	0	0 (0 0%)

Numerical Aberrations 3 hour exposure in the presence of S9

	Cells counted	Endoreduplication	Hyperdiploid	Polyploid	Total
Control	201	0	0	1	1 (0 5%)
APO	172	0	0	3	3 (1 7%)
APO + GSH	204	1	0	3	4 (2 0%)
GSH	201	1	0	0	1 (0 5%)

APPEARS THIS WAY
ON ORIGINAL

Study to Evaluate the Chromosome Damaging Potential of Apomorphine HCl by Its Effects on Cultured Human Lymphocytes Using an In Vitro Cytogenetics Assay

Study no — **Study No** 1/ —
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 16 / Page 71**
Conducting laboratory and location —
Date of study initiation **January 16, 1991**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **19190,** — **% pure**
Formulation/vehicle **distilled water**

Methods

Strains/species/cell line lymphocytes from a single male donor
Dose selection criteria
Basis of dose selection cytotoxicity (based on mitotic index)
Range finding studies yes
Test agent stability
Metabolic activation system Aroclor 1254 induced male Wistar rat hepatic S9
Controls
Vehicle distilled water
Negative controls
Positive controls methyl methanesulphonate (-S9), cyclophosphamide (+S9)
Comments
Exposure conditions
Incubation and sampling times 20 and 44 hours (-S9), 3 hours expression with 17 or 41 hour recovery
Doses used in definitive study
Study design
Analysis
No. of replicates duplicate (quadruplicate in solvent contorol)
Counting method
Criteria for positive results statistically significant increase in aberrations (not counting gaps) and above historical control levels

Key findings

Apomorphine was strongly positive in the presence of S9 at 356 ug/ml and above, it also induced a significant increase at 47.51 ug/ml in the absence of S9, but not at 63.35 or 84.47 ug/ml. The report suggests that the positive result in the absence of S9 was not biologically significant since there was not increase at higher doses. On the other hand, apomorphine was positive in the absence of S9 in other assays. That suggests that the positive results are more likely to be biologically significant in this case. Due to the clearly positive results in the 20 hour cultures, the 44 hour sampling time was not analysed.

20 Hour Expression without S9

Dose	Mitotic Index			Cells	%Cell Aberrations		Aberration Type
ug/ml	A	B	Mean	counted	-Gaps	+Gaps	(cells with aberration)
Control			6.3	200	1.0%	1.5%	Chromatid deletion (2)
47.51			5.0	200	**4.5%**	10.5%	Chromosome deletion (1) Chromatid deletion (9)
63.35			3.5	200	3.0%	6.0%	Chromosome deletion (3) Chromatid deletion (3)
84.47			2.8	200	1.5%	7.5%	Chromatid deletion (4)
MMS	---	---	---	100	27.0%	32.0%	Chromosome deletion (9) Chromatid deletion (10) Chromatid exchange (30) Other (4)

Other includes pulverized cells and cells with 7 or more aberrations

3 Hour Expression, 17 Hour Recovery with S9- Experiment A

Dose	Mitotic Index			Cells	%Cell Aberrations		Aberration Type
	A	B	Mean	counted	-Gaps	+Gaps	(cells with aberration)
Control			4.4	200	0.0%	1.5%	---
356			4.9	200	**6.0%**	7.5%	Chromosome deletion (7) Chromatid deletion (7) Chromatid exchange (1)
474.6			3.0	200	**14.5%**	24.5%	Chromosome deletion (10) Chromosome exchange (1) Chromatid deletion (16) Chromatid exchange (5) Other (1)
632.8			1.6	168	**32.7%**	44.6%	Chromosome deletion (40) Chromatid deletion (51) Chromatid exchange (5) Other (1)
Cyclophosphamide	---	---	---	50	**30.0%**	48.0%	Chromosome deletion (5) Chromatid deletion (14) Chromatid exchange (2)

Numerical Aberrations -S9 20 hour exposure

	Cells counted	Endoreduplication	Hyperdiploid	Polyploid	Total
Control	201	0	0	0	0 (0 0%)
47 51	201	0	1	0	1 (0 5%)
63 35	200	0	0	0	0 (0 0%)
84 47	201	0	0	1	1 (0 5%)
MMS	50	0	0	0	0 (0 0%)

Numerical Aberrations +S9 3 hour exposure, 17 hour recovery

	Cells counted	Endoreduplication	Hyperdiploid	Polyploid	Total
Control	201	0	0	1	1 (0 5%)
356	206	0	2	4	6 (2 9%)
474 6	202	0	1	1	2 (1 0%)
632 8	172	1	2	1	4 (2 3%)
CPA	50	0	0	0	0 (0 0%)

APPEARS THIS WAY
ON ORIGINAL

Study to Evaluate the Chromosome Damaging Potential of Apomorphine HCl by Its Effects on Cultured Human Lymphocytes the Absence and Presence of Ascorbic Acid and Glutathione

Study no — **Study No** 27/ —
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 16 / Page 102**
Conducting laboratory and location —
Date of study initiation **March 3, 1993**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **103120, — % pure**
Formulation/vehicle **distilled water**

Methods

Strains/species/cell line lymhocytes from a single male donor
Dose selection criteria
Basis of dose selection effects on mitotic index
Range finding studies
Test agent stability
Metabolic activation system None
Controls
Vehicle
Negative controls None
Positive controls 4-Nitroquinoline-1-oxide
Comments
Exposure conditions
Incubation and sampling times 44 hours
Doses used in definitive study
Apomorphine (APO)- 113 1 ug/ml
Ascorbic Acid (ASC)- 1 mM
Glutathione (GSH)- 200 ug/ml
Study design
Analysis
No of replicates Single (Duplicate for ascorbic acid)
Counting method
Criteria for positive results

Key findings

Ascorbic acid potentiated the genotoxicity of apomorphine in cultured lymphocytes Glutathione appeared to decrease this potentiation

20 Hour Expression without S9

Dose	Mitotic Index			Cells	%Cell Aberrations		Aberration Type
ug/ml	A	B	Mean	counted	-Gaps	+Gaps	(cells with aberration)
ASC			4 4	400	3 0%	4 8%	Chromosome deletion (8) Chromatid deletion (4)
APO		---	4 0	200	**6 0%**	8 0%	Chromosome deletion (6) Chromatid deletion (6)
APO + ASC		---	4 0	125	**15 2%**	24 8%	Chromosome deletion (6) Chromatid deletion (13)
APO + ASC + GSH		---	1 7	136	**6 6%**	11 8%	Chromosome deletion (5) Chromatid deletion (4)
NQO	---	---	---	50	**8 8%**	16 0%	Chromosome deletion (4) Chromatid deletion (2)

Other includes pulverized cells and cells with 7 or more aberrations

Numerical Aberrations -S9 44 hour exposure

	Cells counted	Endoreduplication	Hyperdiploid	Polyploid	Total
ASC	405	0	1	4	5 (1 2%)
APO	206	1	1	4	6 (2 9%)
APO + ASC	133	2	0	6	8 (6 0%)
APO + ASC + GSH	142	2	0	4	6 (4 2%)
NQO	53	0	2	1	3 (5 7%)

APPEARS THIS WAY
ON ORIGINAL

Study to Evaluate the Potential of Apomorphine to Induce Micronuclei in the Polychromatic Erythrocytes of CD-1 Mice

Study no 27/ —
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 16 / Page 124**
Conducting laboratory and location —
Date of study initiation **July 16, 1992**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **200863, — % pure**
Formulation/vehicle **sodium metabisulfite (1 mg/ml) in water**

Methods

Strains/species/cell line Mouse, CD-1
Dose selection criteria
Basis of dose selection Lethality 2 days after two daily IV injections
Range finding studies

Lethality

Dose (mg/kg/day)	Males	Females
9 8	0/3	0/3
15 1	0/3	0/3
23 2	0/3	0/3
35 7	1/3	2/3
54 9	3/3	2/3
84 5	ND	3/3

Test agent stability
Metabolic activation system Not applicable
Controls
Vehicle yes
Negative controls
Positive controls Cyclophosphamide 40 mg/kg
Comments
Exposure conditions
Incubation and sampling times 24 and 48 hours post 2nd dose
Doses used in definitive study 0, 6 75, 13 5, 27 mg/kg IV
Study design PCE isolated from bone marrow
Analysis
No of replicates 5 mice/sex/dose, 2000 PCE/mouse
Counting method
Criteria for positive results Statistically significant increase above

Key findings
Apomorphine did not increase the incidence of micronuclei

Hour	Dose	Males		Females		Total
		PCE/NCE	MN/ 1000 PCE	PCE/NCE	MN/ 1000 PCE	MN/ 1000 PCE
24	0	1 31	0 20	1 28	0 50	0 35
	6 75	1 30	0 70	1 24	0 50	0 55
	13 5	1 13	0 25	1 43	0 50	0 39
	27	1 05	0 37	1 39	0 20	0 27
	CPA	1 11	13 13	1 22	18 27	15 70
48	0	1 13	0 49	1 32	0 50	0 50
	6 75	1 24	0 20	1 21	0 30	0 25
	13 5	1 24	0 00	1 64	0 60	0 33
	27	1 29	0 87	1 43	0 50	0 66

Historical vehicle control data

Sex		Group Mean Ratio PCE/NCE*	Group Mean Frequency of Micronucleated PCE (per 1000)*	Animals (%) with 0,1 (or more) micronuclei (per 1000) **				
				0	1	2	3	4
Males	Mean	1 13	0 92					
	Range			35	40	19	6	0 4
Females	Mean	1 15	0 76					
	Range			45	39	12	4	0 4

* average of group means from 26 consecutive studies at 12 09 1991
data from all sampling times are combined

** individual animal profile based on data from 234 males and females

Figure 8, from page 27 of Report 27/

Study to Evaluate the Potential of Apomorphine Hydrochloride to Induce Micronuclei in the Polychromatic Erythrocytes of CD-1 Mice

Study no 1/ —
Study type (if not reflected in title)
Volume #, and page #: **Section 5 / Volume 16 / Page 156**
Conducting laboratory and location —
Date of study initiation
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **19190**
Formulation/vehicle **saline (0 9% NaCl in water)**

Methods

Strains/species/cell line Mouse, CD-1
Dose selection criteria
Basis of dose selection Lethality 2 days after two daily injections
Range finding studies at 55 3, mice died after first injection

Lethality

Dose (mg/kg/day)	Males	Females
2 7	0/3	0/3
4 2	0/3	0/3
6 4	0/3	0/3
9 9	0/3	0/3
15 2	0/3	0/3
23 3	0/3	0/3
35 9	3/3	1/3
55 3	3/3	3/3

Test agent stability
Metabolic activation system Not applicable
Controls
Vehicle Yes
Negative controls
Positive controls Cyclophosphamide 40 mg/kg
Comments
Exposure conditions
Incubation and sampling times 24 and 48 hours post 2nd dose
Doses used in definitive study 0, 5 85, 11 7, 23 4 mg/kg, IV
Study design PCE isolated from bone marrow
Analysis
No of replicates 4 mice/sex/dose, 2000 PCE/mouse
Counting method
Criteria for positive results Statistically significant increase above control

Key findings
Apomorphine did not increase the incidence of micronuclei

Hour	Dose	Males		Females		Total
		PCE/NCE	MN/ 1000 PCE	PCE/NCE	MN/ 1000 PCE	MN/ 1000 PCE
24	0	1 23	1 10	1 17	0 98	1 04
	5 85	1 26	0 00	1 25	0 37	0 18
	11 7	1 21	0 50	1 02	0 12	0 31
	23 4	1 36	0 12	1 21	0 25	0 19
	CPA	1 37	16 03	1 12	14 92	15 48
48	0	0 80	0 75	1 16	0 62	0 69
	5 85	0 93	0 75	1 56	0 62	0 69
	11 7	0 89	0 50	1 13	0 62	0 56
	23 4	0 95	0 62	1 09	0 62	0 62

Study to Evaluate the Potential of Apomorphine to Induce Unscheduled DNA Synthesis in Rat Liver Using an In Vivo/In Vitro Procedure

Study no '27 —
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 16 / Page 190**
Conducting laboratory and location —
Date of study initiation **July 9, 1992**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity **200863, — % pure**
Formulation/vehicle **saline**

Methods

Strains/species/cell line Rat, wistar, male
Dose selection criteria
Basis of dose selection lethality
Range finding studies 1/3 rats died at 50 mg/kg IV, 0/3 at 25 mg/kg
Test agent stability
Metabolic activation system Not applicable
Controls
Vehicle Yes
Negative controls
Positive controls 2-acetoaminfluorene (75 mg/kg), dimethylnitrosamine (10 mg/kg)
Comments
Exposure conditions
Incubation and sampling times 2-4 hours post injection, 12-14 hours post injection
Doses used in definitive study 9 487, 30 mg/kg IV
Study design hepatocytes were isolated from apomorphine treated rats, cells were incubated with 10 uCi/ml tritiated thymidine for 4 hours followed by 24 hours with unlabelled thymidine, autoradiography was performed and the number of grains were counted
Analysis
No of replicates 6 male rats/dose, 100 cells/rat
Counting method grains counted —
—
Criteria for positive results at least 20% of cells had net grain counts of at least 5

Key findings

Apomorphine was inactive in this assay

TABLE 1: Group mean net grain count values

Experiment 1, 12-14 hours

Dose (mg/kg)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≥ 5)	
	mean	SD	mean	SD	mean	SD
0 Na met	-0 2	0.3	7.7	3 0	1.4	2.2
9 487	-0 4	0 2	5 3	0 3	1 2	1 1
30	-0 7	0 5	6.0	-	0 4	0 9
75 2-AAF	12 4	2.6	13 5	2 1	79 4	12 9

Experiment 2, 2-4 hours

Dose (mg/kg)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≥ 5)	
	mean	SD	mean	SD	mean	SD
0 Na met	-0.1	0 1	5.8	0.6	1.0	1.7
9.487	-0 2	0.3	7.2	0.7	0 4	0.5
30	-0 2	0 2	5 9	1.0	0 8	0 8
10 DMN	5 6	0.8	8.9	0.8	51 8	8 9

Figure 9, from page 20 or Report 27/ —

Study to Evaluate the Potential of Apomorphine Hydrochloride to Induce Unscheduled DNA Synthesis in Rat Liver Using an In Vivo/In Vitro Procedure

Study no 1/1 ——
Study type (if not reflected in title)
Volume #, and page # **Section 5 / Volume 16 / Page 231**
Conducting laboratory and location ——
Date of study initiation **June 6, 1991**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity
Formulation/vehicle **saline**

Methods

Strains/species/cell line Rat, wistar, male
Dose selection criteria
Basis of dose selection lethality
Range finding studies 3/3 rats died at 50 and 100 mg/kg IV, 0/3 at 25 mg/kg
Test agent stability
Metabolic activation system Not applicable
Controls
Vehicle Yes
Negative controls
Positive controls 2-acetoaminfluorene (50 mg/kg), dimethylnitrosamine (10 mg/kg)
Comments
Exposure conditions
Incubation and sampling times 2-4 hours post injection, 12-14 hours post injection
Doses used in definitive study 7 9, 25 mg/kg IV
Study design hepatocytes were isolated from apomorphine treated rats, cells were incubated with 10 uCi/ml tritiated thymidine for 4 hours followed by 24 hours with unlabelled thymidine, autoradiography was performed and the number of grains were counted
Analysis
No of replicates 5 male rats/dose, 50 cells/rat
Counting method grains counted ——
——
Criteria for positive results at least 20% of cells had net grain counts of at least 5

Key findings
Apomorphine was inactive in this assay

TABLE 1: Group mean net grain count values

2-3 hours sacrifice time

Dose (mg/kg)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≥ 5)	
	mean	SD	mean	SD	mean	SD
0 SALINE	-0.3	0.3	7.7	2.7	2.4	3.3
7 9	-0.8	0 7	11 0	9.5	3 6	4 3
25	-0 7	0 4	5 9	1.2	0.8	1.1
10 DMN	7 5	3.5	10.5	2.9	56 8	23 3

12-13 hours sacrifice time

Dose (mg/kg)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≥ 5)	
	mean	SD	mean	SD	mean	SD
0 SALINE	-1.2	0.6	0	-	-	-
7.9	-1.7	0 7	0	-	-	-
25	-1.5	0 4	0	-	-	-
50 2-AAF	10.9	2.7	11 4	2 4	88.8	13.2

Figure 10, from page 21 of Report 1/

CARCINOGENICITY

No studies were submitted

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

No studies were submitted

SPECIAL TOXICOLOGY STUDIES

Study of Local Irritative Effects of 3 Apomorphine Formulations 2 and 7 Days after Intramuscular Injection in Rabbits

Study no —91189, Study No 90092
Volume #, and page # **Section 5 / Volume 15 / Page 1**
Conducting laboratory and location —
Date of study initiation **January 22, 1991**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity
Formulation/vehicle

Ingredient	Formulation 1	Formulation 2	Formulation 3
Apomorphine HCl	10 0 mg/ml	10 0 mg/ml	10 0 mg/ml
Sodium Bisulfite	1 0 mg/ml	1 0 mg/ml	1 0 mg/ml
Methylparahydroxybenzoate	1 0 mg/ml	1 0 mg/ml	1 0 mg/ml
Benzyl Alcohol	10 0 mg/ml	10 0 mg/ml	10 0 mg/ml
Sodium Edetate	0 1 mg/ml	0 1 mg/ml	0 1 mg/ml
Sodium Chloride	4 5 mg/ml	3 0 mg/ml	---
Acetate Buffer	---	0 15 ml/ml	0 45 ml/ml
Distilled Water	As needed	As needed	As needed

Methods
Male rabbits (3/group) were injected IM with 0 5 ml of one of the apomorphine formulations and sacrificed 2 or 7 days post injection Total apomorphine dose was 5 mg/rabbitThe negative and positive controls were 0 9% aqueous sodium chloride and 0 75% acetic acid, respectively The same rabbits were used for positive (left side) and negative (right side) control injections Injection sites were evaluated for signs of irritation

Results

TABLE 1: MACROSCOPIC AND MICROSCOPIC FINDINGS 2 DAYS AFTER INTRAMUSCULAR INJECTION IN RABBITS

Testpreparation		Animal no	Macroscopic Findings	Microscopic Findings
NaCl (0 9%)	R	001	12x6 mm area of hemorrhage	Focal necrosis with macrophage infiltrate
NaCl (0 9%)	R	002	10x1 mm white line	Small focus of macrophage
NaCl (0 9%)	R	003	10x1 mm hemorrhage	N A
Apomorphine 1	R	007	18x7 mm hemorrhagic area	Patchy necrosis with macrophage infiltration and proliferating fibroblasts
Apomorphine 1	R	008	16x14 mm irregular grey foci hemorrhagic area	Same as above plus PMN infiltrate
Apomorphine 1	R	009	16x7 mm white to hemorrhagic zone	Same as above
Apomorphine 2	R	013	18x11 mm deep red hemorrhagic area with some grey foci	Zone of necrosis surrounded by edema macrophage infiltrate PMN and proliferating fibroblasts
Apomorphine 2	R	014	5x4 mm red-grey zone	Zone of patchy necrosis with macrophage infiltrate
Apomorphine 2	R	015	20x7 mm deep red-grey zone	Zone of necrosis surrounded by edema macrophage infiltrate and proliferating fibroblasts
Apomorphine 3	R	019	17x7 mm deep red-grey zone	Zone of necrosis surrounded diffusely by edema macrophage fibroblast and PMN
Apomorphine 3	R	020	25x15 mm red-grey zone	Same as above
Apomorphine 3	R	021	17x11 mm deep red-grey zone	Same as above
Acetic Acid (0 75%)	L	001	20x7 mm hemorrhagic area with some grey in middle	Demarcated zone of necrotic tissue surrounded by macrophage and proliferating fibroblasts
Acetic Acid (0 75%)	L	002	23x9 mm hemorrhagic area with grey foci in area	Same as above

Figure 11, from page 8 of Report — 911189

TABLE 1 (con't) Study No 90092

Testpreparation		Animal no	Macroscopic Findings	Microscopic Findings
Acetic Acid (0 75%)	L	003	12x12 mm hemorrhagic area within are some greyfoci	Diffuse area of necrosis edema granulocytes macrophage infiltrations and proliferating fibroblasts

Figure 12, from page 9 of Report — 911189

TABLE 2: MACROSCOPIC AND MICROSCOPIC FINDINGS 7 DAYS AFTER INTRAMUSCULAR INJECTION IN RABBITS

Testpreparation		Animal No.	Macroscopic Findings	Microscopic Findings
NaCl (0 9%)	R	004	N A	-
NaCl (0 9%)	R	005	N A	-
NaCl (0 9%)	R	006	N A	-
Apomorphine 1	R	010	17x6 mm grey zone just under fascia	Zone of necrosis surrounded by macrophage granulation tissue and regenerating myoblasts
Apomorphine 1	R	011	20x6 mm grey zone just under fascia	As above
Apomorphine 1	R	012	N A	-
Apomorphine 2	R	016	22x4 mm red-grey zone	As found in animal 010
Apomorphine 2	R	017	17x6 mm red-grey zone	As above
Apomorphine 2	R	018	15x4 mm brown-grey zone	As above
Apomorphine 3	R	022	20x7 mm grey-pink zone	As above
Apomorphine 3	R	023	18x7 mm grey friable zone	As above
Apomorphine 3	R	024	18x7 mm grey friable zone	As above
Acetic Acid (0 75%)	L	004	20x4 mm grey-pink zone	As above
Acetic Acid (0 75%)	L	005	16x7 mm red-pink-grey zone	As above
Acefic Acid (0 75%)	L	006	20x3 mm red-grey zone red outlines zone	As above

Figure 13, from page 10 of Report — 911189

Key study findings

None of the formulations tested match the proposed clinical formulation This study has practically no impact on the approvability of the drug product

The three formulations of apomorphine had similar irritative properties as the positive control acetic acid

Apomorphine - Muscle Irritation Study in Rabbits (Preparation J, K, L, M and N)

Study no — Report Lab No 12965
Volume #, and page # Section 5 / Volume 15 / Page 16
Conducting laboratory and location·
Date of study initiation April 12, 1991
GLP compliance Yes
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity
Formulation/vehicle

Ingredient	Form J	Form K	Form L	Form M	Form N
Apomorphine HCl	10 0 mg/ml	10 0 mg/ml	10 0 mg/ml	10 0 mg/ml	10 0 mg/ml
Sodium Bisulfite	1 0 mg/ml	1 0 mg/ml	1 0 mg/ml	1 0 mg/ml	1 0 mg/ml
Methyl-para-oxybenzoate	1 0 mg/ml	1 0 mg/ml	1 0 mg/ml	1 0 mg/ml	---
Benzyl Alcohol	10 0 mg/ml	10 0 mg/ml	5 0 mg/ml	10 0 mg/ml	---
Sodium Edetate	0 1 mg/ml	0 1 mg/ml	0 1 mg/ml	---	---
Glycerin	11 5 mg/ml	0 8 mg/ml	11 5 mg/ml	11 5 mg/ml	11 5 mg/ml
Acetate Buffer	---	100 mg/ml	---	---	---
Distilled Water	As needed	As needed	As needed	As needed	As needed
pH	3 8	3 8	3 7	3 7	3 7

Methods
Male rabbits (6/group) were injected IM with 0 5 ml of one of the apomorphine formulations and sacrificed 3 days post injection Total apomorphine dose was 5 mg/rabbit Injection sites were evaluated for macroscopic changes and muscle creatinine kinase (CK) levels The contralateral longissimus dorsi muscle was used as control for creatine kinase determination

Results
Macroscopic Observations

Ingredient	Form J	Form K	Form L	Form M	Form N
Irregular Necrosis	6/6	6/6	5/6	5/6	0/6
Regular Necrosis	0/6	0/6	1/6	1/6	0/6
Hemorrhage	5/6	6/6	5/6	5/6	3/6
Petechial hemorrhages	0/6	0/6	0/6	0/6	3/6
Muscle CK content (% of control muscle)	57%	46%	64%	56%	89%

Key study findings
None of the formulations tested match the proposed clinical formulation This study has practically no impact on the approvability of the drug product
Formulation N was the least irritating of the formulations

Apomorphine (Preparation O, P, R and S) - Muscle Irritation Study in Rabbits

Study no [redacted] Report Lab No 12965
Volume #, and page # **Section 5 / Volume 15 / Page 36**
Conducting laboratory and location [redacted]
Date of study initiation **September 24, 1991**
GLP compliance **Yes**
QA reports yes (X) no ( )
Drug, lot #, radiolabel, and % purity
Formulation/vehicle

Ingredient	Form O	Form P	Form R	Form S
Apomorphine HCl	10 0 mg/ml	10 0 mg/ml	10 0 mg/ml	10 0 mg/ml
Sodium Bisulfite	1 0 mg/ml	1 0 mg/ml	1 0 mg/ml	1 0 mg/ml
Methyl-para-Oxybenzoate	1 0 mg/ml	1 0 mg/ml	---	---
Benzyl Alcohol	10 0 mg/ml	7 5 mg/ml	10 0 mg/ml	---
Sodium Edetate	0 1 mg/ml	0 1 mg/ml	0 1 mg/ml	---
Glycerin	11 5 mg/ml	11 5 mg/ml	11 5 mg/ml	---
Distilled Water	As needed	As needed	As needed	As needed
pH	3 8	3 7	3 7	3 8

Methods
Male rabbits (6/group) were injected IM with 0 5 ml of one of the apomorphine formulations and sacrificed 3 days post injection Total apomorphine dose was 5 mg/rabbit Injection sites were evaluated for macroscopic changes and muscle creatinine kinase (CK) levels The contralateral longissimus dorsi muscle was used as control for creatine kinase determination

Results
Macroscopic Observations

Ingredient	Form O	Form P	Form R	Form S
Diffuse Necrosis	3/6	6/6	6/6	0/6
Grey Stripes	3/6	6/6	1/6	2/6
Few Grey Stripes	0/6	0/6	0/6	4/6
Hemorrhage	6/6	5/6	6/6	6/6
Muscle CK content (% of control muscle)	73%	72%	79%	79%

Key study findings
Formulation S, which matches the proposed vial clinical formulation, was the least irritating of the formulations

OVERALL SUMMARY AND EVALUATION

Introduction

Parkinson's disease is a neurodegenerative disease characterized by bradykinesia, muscular rigidity, resting tremors and postural instability. If left untreated, it will 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. Although numerous studies have been conducted on the pharmacology and potential clinical utility of apomorphine, relatively few of these studies have addressed the preclinical issues that are necessary to address the potential adverse effects of the drug when used clinically. The studies in the open scientific literature that address these preclinical issues lack the details necessary to make a full evaluation of the data. These studies may provide suggestive evidence of a potential problem, but in most circumstances, they do not provide quality of data needed to make a judgement on potential risks. The sponsor has placed great reliance on this literature to support their application. Where appropriate, this reviewer will discuss the data from the open literature. This reviewer has supplemented the sponsor's review of the open literature by conducting a Pub Med literature search covering the period from January 2002 through April 2003. This would identify any additional studies published since the sponsor submitted their preclinical package in May 2002. The sponsor has obtained preclinical reports from other companies to support their application. In addition, the sponsor has conducted additional studies to fill in some of the data gaps. The sponsor has requested that the requirement for carcinogenicity studies be waived. The sponsor has also requested that the requirement for reproductive toxicity studies be waived. This section will integrate the data from the submitted studies and the open literature to assess the adequacy of the preclinical studies to support the approval of the drug product. Emphasis is placed on adverse effects that are difficult to detect clinically.

Safety Evaluation

PHARMACOLOGY STUDIES

The purpose of the pharmacology section is to identify how the drug affects the body. These studies are useful for identifying potential mechanisms of action. These data can also aid in the assessment of interactions between therapies. The sponsor has not conducted any pharmacology studies on apomorphine, although they have submitted papers from the open scientific literature concerning the pharmacology of apomorphine.

The sponsor submitted several papers which indicate that apomorphine is active in animal models of Parkinson's disease In these studies, a Parkinson's-like syndrome is induced in rats or monkeys by treating them with 6-hydroxydopamine or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Most of the studies used unilateral lesions and examined contralateral rotations following apomorphine administration As would be expected from a dopaminergic agonist, apomorphine demonstrated activity in these animal models of Parkinson's disease In rats pretreated with 6-hydroxydopamine, the effective doses ranged from 0 018 mg/kg to 1 mg/kg by subcutaneous injection In monkeys pretreated with MPTP, the effective doses ranged from 0 05 to 0 5 mg/kg by subcutaneous injection

Apomorphine is an agonist at the dopamine D1, D2, D3, D4, and D5 receptors The D3, D4, and D5 receptors are presynaptic and probably do not contribute to the therapeutic efficacy of apomorphine The available data suggest that apomorphine has a greater affinity for the dopamine D2 receptor than the dopamine D1 receptor The binding of apomorphine to other receptors has been examined [1],[2],[3] These studies suggest that apomorphine can interact with a variety of other receptors With the exception of the histamine receptor (from guinea pig cerebellum) and the adrenergic alpha-2D (rat cortex), this study used cloned human receptors transfected into CHO cells The following table provides a listing of the pKi (-log Ki values), Ki, pEC50 (-log EC50 values), EC50 and Emax (percent maximum stimulation of native agonist) for various binding sites

[1] Millan, MJ, L Maiofiss, D Cussac, V Audinot, J-A Boutin and A Newman-Tancredi (2002) Differential Actions of antiparkinson agents at multiple classes of monoaminergic receptor I A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes J Pharmacol Exp Ther 303(2) 791-804

[2] Newman-Tancredi, A, D Cussac, V Audinot, J-P Nicolas, F de Ceuninck, J-A Boutin and MJ Millan (2002) Differential Actions of antiparkinson agents at multiple classes of monoaminergic receptor II Agonist and antagonist properties at subtypes of dopamine D2-like receptor and alpha-1/alpha-2 adrenoceptor J Pharmacol Exp Ther 303(2) 805-814

[3] Newman-Tancredi, A, D Cussac, Y Quentric, M Touzard, L Verriele, N Carpentier and MJ Millan (2002) Differential Actions of antiparkinson agents at multiple classes of monoaminergic receptor II Agonist and antagonist properties at serotonin, 5 HT1 and 5-HT2, receptor subtypes J Pharmacol Exp Ther 303(2) 815-822

In Vitro Binding for Apomorphine					
Receptor	pKi	Ki (nM)	PEC50	EC50	Emax
Dopamine D1	6.43	370	---	---	---
Dopamine D2S	7.46	35	7.71	19	79%
Dopamine D2L	7.08	83	7.66	22	53%
Dopamine D3	7.59	26	7.93	12	82%
Dopamine D4	8.36	4.4	8.23	5.9	45%
Dopamine D5	7.83	15	---	---	---
Adrenergic alpha-1A	5.70	2,000	---	---	---
Adrenergic alpha-1B	6.17	680	---	---	---
Adrenergic alpha-1D	7.19	65	---	---	---
Adrenergic alpha-2D	6.49	320	---	---	---
Adrenergic alpha-2A	6.85	140	6.92	120	16
Adrenergic alpha-2B	7.18	66	**6.84**	**140**	0
Adrenergic alpha-2C	7.44	36	**6.55**	**280**	0
Adrenergic beta-1	<5	>10,000	---	---	---
Adrenergic beta-2	<5	>10,000	---	---	---
5-HT1A	6.93	120	5.92	1,200	35
5-HT1B	5.53	3,000	<5	>10,000	0
5-HT1D	5.91	1,200	<5	>10,000	0
5-HT2A	6.92	120	**6.43**	**370**	0
5-HT2B	6.88	130	**6.18**	**660**	0
5-HT2C	6.99	100	**6.06**	**870**	0
Histamine H1	<5	>10,000	---	---	---
Values in **Bold** indicate pKb values (indicator of antagonist activity) --- means not tested					

These data indicate that apomorphine is an agonist at the dopamine D3, D4 and D5 sites as well as the D1 and D2 sites. These data also suggest that apomorphine may bind to adrenergic alpha-1 and alpha-2 receptors. Although these studies covered a variety of receptor sites, there are a variety of other receptor sites that would be useful to examine. This reviewer is particularly interested in interactions with adenosine receptors, which have been proposed as an alternative target for therapy in Parkinson's disease.

This reviewer considers the pharmacology studies adequate to support approval.

PHARMACOKINETICS

The purpose of the pharmacokinetic evaluation is to determine whether the pharmacokinetics of the drug substance is similar in humans and the preclinical species. If humans make a metabolite that is not made in preclinical species, then the preclinical studies may not identify all the potential toxic effects of a compound. Likewise, if an animal makes a metabolite that is not present in humans, then it may predict toxicities that may not occur in humans. Concurrence in the formation of metabolites offers assurances that the preclinical studies are valid models for human exposures. The sponsor has submitted several pharmacokinetic studies as well as scientific papers related to the pharmacokinetics of apomorphine.

It is known from the scientific literature that apomorphine has poor oral bioavailability (about 4%),[4] so it is generally administered by subcutaneous injection. The sponsor's pharmacokinetic/toxicokinetic studies demonstrate that apomorphine is rapidly absorbed into the blood stream following subcutaneous injection (Tmax=15 minutes). No data on the tissue distribution among the internal organs (eg, the liver, kidney, etc) were provided, although several studies in the scientific literature have found that apomorphine is rapidly taken up into the brain.[5,6] The available data suggest that apomorphine is rapidly eliminated from the body (plasma half-life is about 15 minutes in rats and monkeys). In the toxicokinetic studies, the apomorphine plasma concentration was less than the level of quantification (5 ng/ml) 90 minutes after subcutaneous injection of 0.75 mg/kg to rats. In rats and monkeys, the urine is the primary route of elimination, although the feces also accounts for about a third of excreted apomorphine.

No data were identified on the metabolism of apomorphine in monkeys. The in vivo metabolism in rats and mice has not been elucidated in the published literature. Glucoronidation is a major metabolic pathway, but whether the substrate is the parent compound or a metabolite is uncertain. The sponsor suggests that auto-oxidation may be a key metabolic pathway citing a paper by Sam et al. (1994).[7] In this study, the investigators incubated apomorphine in plasma and measured the disappearance of apomorphine. They found that apomorphine disappeared with a half-life of approximately 39 minutes. The disappearance of apomorphine was inhibited by mercaptoethanol (0.01% v/v), but not by sodium bisulfite (1% w/v). Since plasma has multiple enzymes which may be able to interact with apomorphine, this does not establish that the disappearance of apomorphine was due to auto-oxidation. In addition, mercaptoethanol, which interacts with sulfhydryl groups, could inhibit enzymes responsible for apomorphine metabolism. This reviewer has similar concerns about the report of auto-oxidation in whole blood in the Van der Geest et al. (1998) study, also cited by the sponsor.[8]

In summary, the pharmacokinetic studies suggest that apomorphine is rapidly absorbed following subcutaneous injection. It is eliminated from the blood rapidly in vivo. The metabolism of apomorphine has not been elucidated in monkeys, rats or mice. It is known that apomorphine or its metabolites forms conjugates with glucoronic acid and sulfate. The Biopharmacology reviewer (Dr. John Duan) has indicated that the sponsor has not established the pattern of metabolism in humans. Elimination is via the urine and feces.

This reviewer recommends that a mass balance study be conducted in mice, rats and monkeys to determine the metabolism of apomorphine and so help establish the relevance of the preclinical studies. These studies should also be carried out in humans.

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[4] Smith, R.V., A.E. Klein, R.E. Wilcox and W.H. Riffee (1981) Apomorphine bioavailability and effect on stereotyped cage climbing in mice. J. Pharma. Sci. 70(10) 1144-1147.

[5] Symes, A.L., S. Lal and T.L. Sourkes (1975) Effect of catechol-O-methyltransferase inhibitors on brain apomorphine concentrations and stereotyped behaviour in the rat. J. Pharm. Pharmac. 27 947-949.

[6] Burkman, A.M., R.E. Notari and W.K. Van Tyle (1974) Structural effects in drug distribution: comparative pharmacokinetics of apomorphine analogues. J. Pharm. Pharmac. 26 493-507.

[7] Sam, E., P. Augustijns and N. Verbeke (1994) Stability of apomorphine in plasma and its determination by high-performance liquid chromatography with electrochemical detection. J. Chromatog. B 658 311-317.

[8] Van der Geest, T. van Laar, P.P. Kruger, J.M. Gubbens-Stibbe, H.E. Bodde, R.A.C. Roos and M. Danhof (1998) Pharmacokinetics, enantiomer interconversion, and metabolism of R-apomorphine in patients with idiopathic Parkinson's disease. Clin. Neuropharmacol. 21(3) 159-168.

SAFETY PHARMACOLOGY

The purpose of safety pharmacology studies is to detect forms of toxicity that can not be assessed via traditional toxicology studies The sponsor did not submit any safety pharmacology studies, although ECG parameters were evaluated as part of the chronic monkey study The sponsor did submit safety pharmacology studies from the open literature These studies examined the effects of apomorphine on the cardiovascular system (heart rate, blood pressure), the respiratory system and the central nervous system (temperature regulation, endocrine effects, emesis) No studies were submitted on the effects of apomorphine on the gastro-intestinal tract or the kidney

In the chronic monkey study (see page 31), EKG readings were obtained prestudy and during week 39 of the study The week 39 readings were obtained prior to the first dose of the day (that is 16 to 17 hours after the prior dose of apomorphine) (from sponsor's April 1, 2003 submission) No effect on EKG parameters were observed Since the half life of apomorphine is approximately 15 minutes (0 25 hours), the EKG readings were obtained approximately 64 half lifes after the last dose of apomorphine Therefore, the EKG readings were obtained at a time when there was no apomorphine in the system This study is useless for evaluating the potential effects of apomorphine on the cardiac conduction system

Apomorphine, like other dopamine agonists, can reduce blood pressure and affect the heart Apomorphine (0 05 or 0 5 mg/kg IV) caused a decrease in blood pressure and systemic vascular resistance (decreased 20% and 35% from control levels at 15 minutes) in the anesthetized dog [9] This was accompanied by an increase in heart rate (about 20% at 15 minutes), but no changes in QT interval were reported The QTc intervals were not calculated and the data are not sufficient for this reviewer to calculate the QTc intervals No effects were observed in the action potential duration in dog Purkinje cells, but about a 10% increase in the APD90 was observed in dog ventricular muscle cells at 100 nM concentrations and above In another study, 0 2 mg/kg apomorphine IV induced decreased blood pressure and increased heart rate in anesthetized dogs [10] Apomorphine (0 01 and 1 mg/kg IV) was reported to reduce blood pressure in anesthetized rats [11] It was also reported that apomorphine caused a "marked bradycardia (30-100%)", but the data were reported in a confusing manner (oscilloscope photograph) The authors did not attempt to quantify the heart rate or duration of the bradycardia This reviewer is uncertain about the meaning of 100% bradycardia The significance of this reported bradycardia is uncertain Bradycardia associated with hypotension was also reported in cats, but at very low doses (0 005 mg/kg) [12] The sponsor states that Barnett and Firore (1971) showed that high doses (>0 3 mg/kg IV) had a hypertensive effect in cats [13] An examination of the paper revealed that this effect only occurred in spinally transected cats and not in normal cats This finding is unlikely to be applicable to the clinical situation

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[9] Nakayama H, T Nakayama, CA Carnes SM Strauch and RL Hamlin (2001) Electrophysiologic and hemodynamic effects of apomorphine in dogs Toxicol Appl Pharmacol 177 157-161

[10] Montastruc, J, C Guiol, MA Tran, F Lhoste and P Montastruc (1985) Studies on the cardiovascular actions of apomorphine in dogs central versus peripheral mechanisms and role of the adrenal medulla Arch Int Pharmacodyn Ther 277(1) 92-103

[11] Finch, L and G Haeusler (1973) The Cardiovascular effects of apomorpine in the anaesthetized rat Eur J Pharmacol 21 264-270

[12] DeMeyer, JM, WA Buylaert and MG Bogaert (1982) Hypotension and bradycardia by intravenous apomorphine in the anesthetized cat no evidence for a central mechanism Arch Int Pharmacodyn 256(1) 166-167

[13] Barnett, A and JW Firore (1971) Hypotensive effects of apomorphine in anesthetized cats Eur J Pharmacol 14 206-208

In summary, apomorphine can decrease blood pressure in laboratory animals This hypotension can be accompanied by tachycardia at high doses (around 1 mg/kg) or bradycardia at lower doses (at about 0 01 mg/kg) The effects on heart rate may be due to activation of different receptors The potential effects on the ECG parameters have not been adequately evaluated In particular, the potential effects on the QTc interval have not been examined Apomorphine did not affect action potential duration in Purkinje cells, but it did prolong the APD90 in ventricular muscle cells This is suggestive that it might have the potential to affect cardiac electrical system in humans However, the preclinical studies are inadequate to assess the potential effects of apomorphine on EKG parameters

Several studies have examined the effect of apomorphine on respiratory parameters In one study, apomorphine (0 003 to 0 3 mg/kg IV) was administered to alpha-chloralose anesthetized rats [14] At 0 1 and 0 3 mg/kg, a decrease in the respiration rate was observed, but tidal volume was increased The minute volume was slightly reduced In another study, apomorphine increased the respiratory rate and minute volume in halothane-anesthetized rats at doses between 0 3 and 9 mg/kg IP [15] In the absence of data on systemic apomorphine concentration following IP injection, the significance of this study is uncertain In alpha-chloralose (80 mg/kg IV) anesthetized dogs, 0 2 mg/kg apomorphine IV induced "respiratory arrest" within 25 seconds [16] These results are inconsistent with a previous study conducted under identical conditions by the same group which reported that dogs were able to respire spontaneously and that respiratory parameters were not affected by 0 2 mg/kg apomorphine IV treatment [17] In summary, there are limited data on the potential effects of apomorphine on respiratory parameters

Many studies have examined the effects of apomorphine on the central nervous system The effect on behavior (stereotypic behavior, emesis) have been adequately addressed in the submitted preclinical studies and will not be discussed here Apomorphine (0 3-1 25 mg/kg IV) induced hypothermia in mice 30 minutes post injection [18] It also caused hypothermia in rats 15 to 120 minutes post injection at doses between 0 1 and 10 mg/kg SCU [19,20] In contrast, rabbits treated with 1 to 5 mg/kg apomorphine IV developed hyperthermia, which peaked about 1 hours post injection [21] The reason for this discrepancy is uncertain

The available safety pharmacology studies do not provide much support for or against the safety of apomorphine Hypotension is a well known effect of dopaminergic agonists The lack

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[14] Bolme, P, K Fuxe, T Hokfelt and M Goldstein (1977) Studies on the role of dopamine in cardiovascular and respiratory control central versus peripheral mechanisms Adv Biochem Phsychopharmacol 16 281-290

[15] Lundberg, D , GR Breese and RA Mueller (1979) Dopaminergic interaction with the respiratory control system in the rat Eur J Pharmacol 54 153-159

[16] Montastruc, JL, O Rascol and P Montastruc (1992) Naloxone or haloperidol but not yohimbine reverse apomorphine-induced respiratory depression Clin Neuropharmacol 15 404-407

[17] Montastruc, J, C Guiol, MA Tran, F Lhoste and P Montastruc (1985) Studies on the cardiovascular actions of apomorphine in dogs central versus peripheral mechanisms and role of the adrenal medulla Arch Int Pharmacodyn Ther 277(1) 92-103

[18] Moore, NA and MS Axton (1990) The role of multiple dopamine receptors in apomorphine and N-n-propylnorapomorphine-induced climbing and hypothermia Eur J Pharmacol 178 195-201

[19] Varty, GB and GA Higgins (1998) Dopamine agonist-induced hypothermia and disruption of prepulse inhibition evidence for a role of D3 receptors? Behav Pharmacol 9 445-455

[20] Onavi, ES (1993) Apomorphine induced hypothermia, stereotypy and changes in dopamine D2 mRNA expression NeruroReport 4 703-705

[21] Quock RM and A Horita (1974) Apomorphine modification of its hyperthermic effect in rabbits by p-chlorophenylalanine Science 183 539-540

of data on EKG parameters is disturbing However, it is expected that there will be adequate clinical data so that additional preclinical safety pharmacology studies will not be needed If the clinical data suggest that there may a potential problem, the available preclinical data will not provide much guidance in the interpretation of the clinical studies

REPEAT DOSE TOXICITY STUDIES

The toxicity of apomorphine has been examined in a series of 4, 13, 26 (rats only) and 39 (monkeys only) toxicity studies The methods used these studies are broadly consistent across species In all cases, apomorphine was administered in divided doses to mimic human dosing regimen and to permit a higher daily dose than would be possible using a single dose The doses were limited by central nervous system toxicity as evidenced by stereotypic behavior and self mutilation

In the 13 week rat study (see page 12), rats (15/sex/dose) were administered 0, 0 4, 1 0 or 4 0 mg/kg/day in four divided doses (0, 0 1, 0 25 or 1 0 mg/kg/dose) These doses are equivalent to human doses of 0, 3 9, 9 7 or 39 mg/day, assuming a 60 kg patient (based on a mg/m^2 comparison) One treatment related death was observed at 1 mg/kg/day and 5 were observed at 4 mg/kg/day A dose-dependent increase in the frequency and intensity of stereotypic behavior was observed starting at 0 4 mg/kg/day The high dose caused a decrease in body weight in males (-14%), but not in females No significant effects on hematology or clinical chemistry parameters were observed Increased adrenal weight associated with adrenal hypertrophy was observed in the males Increased ovary weight was observed in female rats One out of ten high dose rats had testicular atrophy with small testes

The results of the 26 week rat study (see page 17) were similar to the results of the 13 week study Rats (20/sex/dose) were administered 0, 0 3, 1 0 or 3 0 mg/kg/day in four divided doses (0, 0 075, 0 25 or 0 75 mg/kg/dose) These doses are equivalent to human doses of 0, 2 9, 9 7 or 29 mg/day, assuming a 60 kg patient (based on a mg/m^2 comparison) A dose-dependent increase in stereotypic behavior was observed at 0 3 mg/kg and above A dose-dependent decrease in body weight was observed at 1 and 3 mg/kg/day No significant effects on hematology or clinical chemistry parameters were observed Increased adrenal weight was observed in both males and females, but there was no report of adrenal hypertrophy Increased ovary weight was observed in female rats at 3 mg/kg Increased heart weight was observed in both males and females at 3 mg/kg Five out of 20 male rats at 3 mg/kg had testicular abnormalities including cryptorchid testes (3 rats), hypospermia (1 rat) and degeneration (1 rat) These five rats also had decreased testes weight No testicular abnormalities were observed in 19 control rats The low and mid dose groups were not examined for testicular abnormalities

A general concern about drugs for Parkinson's disease is the potential for interaction with Sinemet (levodopa/carbidopa) Virtually all drugs for advanced Parkinson's disease will be administered in combination with Sinemet To examine the potential for interactions, apomorphine (0, 0 3, 1 0, 3 mg/kg/day) was administered in combination with Sinemet (50 mg/kg [40 mg/kg levodopa / 10 mg/kg carbidopa]) for 13 weeks (see page 21) A dose-dependent increase in stereotypic behavior was observed in apomorphine treated rats Decreased body weight was observed in male apomorphine treated rats (-19% at 3 mg/kg/day) Sinemet did not appear to affect the incidence of stereotypic behavior or body weight decrease when combined with 3 mg/kg/day apomorphine No effects on hematology, clinical chemistry, organ weight or histopathology was observed Sinemet had no adverse effects on treated rats However, the dose (50 mg/kg) is much lower than is generally used in these studies (150 mg/kg), see pramipexole and ropinerole This study should be repeated using a higher dose of Sinemet

In the 13 week monkey study (see page 26), monkeys (4/sex/dose) were administered 0, 0 6, 1 5 or 3 0 mg/kg/day in six divided doses (0, 0 1, 0 25 or 0 5 mg/kg/dose) These doses are equivalent to human doses of 0, 12, 29 or 58 mg/day, assuming a 60 kg patient (based on a mg/m^2 comparison) A dose-dependent increase in behavioral abnormalities (agitation, licking motion, tongue protrusion) was observed at all dose levels No effects on body weight were observed Although there were a variety of organ weight changes, no histological correlates were observed Some decrease in testes weight was observed (8-12%)

In the 39 week monkey study (see page 31), Monkeys (4/sex/dose, 2/sex were used for recovery in control and high dose group) were administered 0, 0 3, or 1 0 mg/kg/day in six divided doses (0, 0 05, 1 7 mg/kg/dose) or a high dose of apomorphine The high dose of apomorphine ranged from 1 25 to 3 0 mg/kg/day (0 21 to 0 5 mg/kg/dose), the monkeys were maintained at a dose of 1 5 mg/kg/day from about week seven of the study through termination The 1 5 mg/kg/day dose is equivalent to a human dose of 29 mg/day, assuming a 60 kg patient (based on a mg/m^2 comparison) One high dose monkey was sacrificed on day 72, when the dose had been raised temporarily to 1 75 mg/kg/day The primary clinical sign was hyperactivity, which is probably similar to the agitation observed in the 13 week study Decreased body weight was observed in high dose males No adverse effects on hematology, clinical chemistry, or histopathology were observed A dose-dependent decrease in relative testes weight was observed (-17%, -47% and -57% in low mid, and high dose monkeys, respectively) The sponsor attributed the decrease in testes weight to juvenile testes, but the incidence of juvenile testes was similar in the treatment groups (1/4, 0/4, 1/4 and 1/3 in control, low, mid and high dose monkeys, respectively) This is probably a treatment related effect It is uncertain why the monkeys tolerated 3 mg/kg/day in the 13 week study, but not in the 39 week study (that dose was maintained for only six days) It may be due to differences between laboratories as to what constitutes an acceptable degree of behavioral changes

In summary, the dose limiting toxicity observed in the repeat dose toxicity studies were CNS signs which reflected exaggerated pharmacological activity One other concern identified in these studies is potential effects on male reproductive capacity A dose-dependent decrease in relative testes weight was observed in the 39 week monkey study Five out of 19 high dose rats in the 26 week study had decreased testes weight associated with testicular abnormalities (no testicular abnormalities were observed in the 20 control rats) In addition, 1/10 high dose rats in the 13 week study had hypospermia (no testicular abnormalities were observed in control rats) Some decreases in testes weight were also observed in monkeys in the 13 week study Segment 1 reproductive toxicity studies incorporating sperm analysis would be needed to more fully evaluate the significance of these findings

Class Effect Retinal Toxicity

Retinal atrophy has been observed in rat carcinogenicity studies with other dopaminergic agonists (pramipexole, ropinerole) There is evidence that this may be a class effect Since carcinogenicity studies were not submitted, it is uncertain whether apomorphine will also cause retinal toxicity Since the retinal toxicity is a class effect, it would be product to assume that apomorphine would also cause retinal toxicity and place an appropriate warning in the label

Genotoxicity Studies

The sponsor has submitted a series of four in vitro bacterial mutagenicity tests, one mouse lymphoma assay, five in vitro chromosomal aberration assays in human lymphocytes, two in vivo mouse micronucleus tests, and two in vivo DNA repair assays

In the four in vitro bacterial mutagenicity tests, apomorphine was consistently positive in Salmonella typhimurium strains TA98 and TA1537 in the presence and absence of metabolic activation The response in the absence of metabolic activation was stronger than in the absence of metabolic activation The response in TA1537 was stronger (10- to 25-fold increase over background) than in TA98 (2- to 4-fold increase over background) Both TA98 and TA1537 are used to detect frameshift mutations Apomorphine was generally negative in strains which test for base-pair substitutions (TA100, TA1535 and Escherichia coli WP2 pKM101 and E coli WP2 uvrA pKM101) Statistically significant increases in the number of revertants were observed in TA100, but the responses were relatively weak (less than two-fold increase over background)

The results in the in vitro mammalian genotoxicity assays are consistent with the results in the bacterial systems Apomorphine was positive in the mouse lymphoma test in the presence and absence of metabolic activation These positive results were observed at doses where there was adequate survival of treated cells Positive results were observed at doses with relative survival of 84% in the absence of metabolic activation and 77% in the presence of metabolic activation An increase was observed in the number of large and small colonies, although the proportion of small colonies increased, suggesting that apomorphine was clastogenic as well as mutagenic In two separate studies in human lymphocytes, apomorphine caused chromosomal aberrations (generally chromosome and chromatid deletions) Positive results were observed at doses at which there were greater than 50% survival (as indicated by changes in mitotic index) in treated cells In some cases, the mitotic index in cultures with positive results were higher than the mitotic index in control cultures This suggests that the genotoxicity of apomorphine is due to a direct effect on the cell rather than through a non-specific cytotoxic pathway

Apomorphine has been examined for mutagenicity in vivo using the mouse micronucleus test and the rat unscheduled DNA synthesis assays In the micronucleus tests, apomorphine was administered as two daily intravenous injections (apomorphine doses were 27 and 23 4 mg/kg) Apomorphine did not increase the number of micronuclei at 24 and 48 hours after the second injection Apomorphine did not increase unscheduled DNA synthesis in rat liver at 25 and 30 mg/kg IV The unscheduled DNA synthesis assay is not considered a sensitive test of in vivo genotoxic potential This reviewer is concerned about the adequacy of the mouse micronucleus tests Apomorphine was administered as a single intravenous injection in these studies, in the clinic, apomorphine will be administered subcutaneously up to four times per day The micronucleus test should be designed to reflect this use This is particularly a concern with apomorphine since the half life of this drug is so short (approximately 15 minutes in rats) that it would be difficult for the bone marrow cells to be exposed long enough to the drug to generate a positive response in this assay It is recommended that the micronucleus test be repeated using four divided doses and the subcutaneous route of exposure

The sponsor suggests that the positive results in the in vitro assays were due to in vitro mechanisms (oxidation, oxygen radical formation), which would not be applicable to the in vivo situation To support this contention, the sponsor cites studies in which apomorphine was incubated with glutathione, superoxide dismutase, or catalase In the Ames assay, the sponsor states that glutathione and superoxide dismutase reduced the mutagenicity of apomorphine However, the effects of superoxide dismutase were inconsistent It had no appreciable effect on the mutagenicity of apomorphine in TA1537 in the presence and absence of metabolic activation (apomorphine doses were 600 and 200 ug/plate, respectively) at concentrations up to 500 units/plate (see page 41) If the mutagenicity of apomorphine was due to in vitro specific mechanisms, one would expect consistency among strains In the same study, glutathione reduced the mutagenicity of apomorphine in the Ames assay However, in another study (see

page 43), Salmonella typhimurium strains TA98, TA100 and TA1537 were pre-incubated with apomorphine for 20 minutes The cells were then washed free of apomorphine and plated for 72 hours in the presence or absence of catalase, superoxide dismutase or glutathione Glutathione reduced the mutagenicity of apomorphine in this study, this suggests that the antimutagenic effect of glutathione in this system is not due to direct interaction with apomorphine This would be consistent with the evidence that glutathione is an antimutagen [22],[23] If the mutagenicity of apomorphine is due to in vitro specific reactions, one would expect similar results the in vitro mammalian genotoxicity assays However, glutathione was ineffective in reducing apomorphine's genotoxicity in the in vitro human lymphocyte chromosomal aberration assay In one study (see page 54), glutathione potentiated the incidence of chromosomal aberrations in apomorphine treated cells (31 2% of cells treated with 200 ug/ml glutathione plus 182 7 ug/ml apomorphine compared to 7 3% in cells treated with apomorphine alone and 1% in control cells) The sponsor states (on page 5-1-125 of their submission) that glutathione reduced the frequency of aberrations in one study (see page 56) Indeed, the incidence of chromosomal aberrations in apomorphine treated cells was reduced (7 8% of cells treated with 200 ug/ml glutathione plus 590 6 ug/ml apomorphine compared to 13 7% in cells treated with apomorphine alone and 1% in control cells) However, the combination of apomorphine and glutathione reduced the mitotic index to less than 10% of control levels (0 4 compare to 5 0 in control culture, apomorphine alone and glutathione alone cultures had mitotic indices of 1 1 and 6 2, respectively) This resulted in a lower number of cells being evaluated in the apomorphine and glutathione combination culture (102 versus 200 in control) The reduction in chromosomal aberrations may have been the result of the cytotoxicity of the combination of agents in this test system Superoxide dismutase reduced the incidence of chromosomal aberrations in cultured human lymphocytes to control levels, but, as noted above, it had no effect on mutagenicity in the Ames assay If the genotoxicity of apomorphine is due to factors unique to the in vitro situation, one would expect for there to be consistency among the results of the various assays

SPECIAL STUDIES

A series of special studies examined the irritation potential of a series of apomorphine formulations Only one of the studies examined the formulation with bisulfite alone, the vial formulation, none of the studies examined the irritancy of the sodium bisulfite/benzyl alcohol mixture used in the cartridge It appears that the sodium bisulfite solution had the least potential for irritation of the various combinations

Impurity/Degradation Issue (As of May 15, 2003)

The sponsor has identified two potential degradation products in the proposed drug product (see Figure 14) These products are formed —

— As of May 15, 2003, the sponsor has proposed a maximum concentration for these degradation products of — in the drug product Since these degradation products have not been tested in preclinical studies, there is potential that additional preclinical studies will be needed to "qualify" these substances ICH guideline Q3B specifies the concentrations of impurities

[22] Waters MD, AL Brady, HF Stack and HE Brockman (1990) Antimutagenicity profiles for some model compounds Mutat Res 238(1) 57-85

[23] Karekar V S Hoshi and SL Shinde (2000) Antimutagenic profile of three antioxidants in the Ames assay and the Drosophila wing spot test Mutat Res 468(2) 183-194

requiring qualification studies, which is dependent on the daily dose of the drug product The sponsor has not specified the dosage schedule for this drug (maximum single dose/number of doses per day) [redacted]

[redacted]

If the specification limits remain at [redacted] then the sponsor will need to conduct additional preclinical studies to qualify the impurities According to ICH guidelines, the necessary toxicology studies would be genotoxicity studies (in vitro bacterial mutagenicity assay and mammalian in vitro genotoxicity assay) and a repeat dose (4-13 week duration) toxicity study in a single species The chemistry reviewer (Dr Thomas Broadbent) has indicated that he will recommend that the specification for the degradation products be lowered to [redacted] If the sponsor is able to meet this specification and the maximum daily dose remains below 40 mg/day, then additional preclinical studies would not be needed to qualify the impurities

[redacted]

[redacted]

Figure 14, Potential Degradation Products

Sponsor Request for a Waiver on the Requirement for Carcinogenicity Studies

The sponsor has not conducted carcinogenicity studies, which are recommended for chronic use drugs of at least two to three years duration The sponsor has requested that the need for carcinogenicity studies be waived for this application (see **Appendix 1 Request for Waiver of Requirement for Carcinogenicity and Reproductive Preclinical Toxicology Studies**) The basis for the sponsor's request is based on two principles First, ICH guidelines do not require pre-approval conduct of carcinogenicity studies Second, carcinogenicity studies have already been conducted on apomorphine These arguments will be discussed below

The sponsor also notes that carcinogenicity studies on apomorphine have been conducted

[redacted], the actual study has not been made part of the public domain [redacted]

[redacted]

ICH guidelines provide a waiver of pre-approval carcinogenicity studies for drugs intended to treat severely debilitating diseases The intent of this exception is that potentially useful therapies should not be delayed in their entry into the market due to the need for carcinogenicity studies However, this guidance does not eliminate the need for carcinogenicity studies The sponsor should be working towards completing these studies Given the strong in vitro genotoxicity results with this drug (see above), this reviewer considers it imperative that the carcinogenic potential of this drug be addressed prior to approval

Sponsor Request for a Waiver on the Requirement for Reproductive Toxicity Studies

The sponsor has not conducted reproductive toxicity studies, which are recommended for drugs intended to treat Parkinson's Disease The sponsor has requested that the need for reproductive toxicity studies be waived for this application (see **Appendix 1 Request for Waiver of Requirement for Carcinogenicity and Reproductive Preclinical Toxicology Studies)** The sponsor makes several argument to support their contention that reproductive toxicity studies are not need for this drug

1 The seriousness of the disease suggests that reproductive toxicity studies should not be required
2 The age and reproductive capacity of the patient population suggest that reproductive toxicity is not a concern
3 Previous literature reports of Segment I studies using subcutaneous apomorphine are sufficient to assess the reproductive toxicity of apomorphine
4 The results of the mouse micronucleus and rat unscheduled DNA synthesis tests were negative

The sponsor suggestion that the seriousness of Parkinson's disease negates the need for reproductive studies is inconsistent with previous policy Comtan, which is indicated for prevention of end of dose "off" episodes was required to conduct reproductive toxicity studies prior to approval In addition, patients have a right to know whether a drug can affect their reproductive toxicity Although Parkinson's disease is a serious disease, patients with Parkinson's disease may wish to reproduce The FDA has approved drugs for severe diseases (e g AIDS, cancer and organ rejection) and required reproductive toxicity studies Patients have a right to know what the potential adverse effects of taking a drug may be on their reproductive capacity

The sponsor suggests that the patients who would use apomorphine would be past the age of reproductive capacity To support this assertion, they state that the average age at diagnosis is 58 They suggest that there is a 15 year progression until patients would reach the late stages of

Parkinson's disease intended to be treated with apomorphine, at which point they would be 73 Even if they wished to reproduce, the debilitating effects of Parkinson's disease would prevent them from being able to have sexual intercourse The sponsor's neglects to consider the range of ages at which Parkinson's disease occurs and the age distribution in their own clinical trials The minimum ages in their four clinical trials were 38, 42, 45 and 46 years of age This suggests that the range of patients who may be taking apomorphine does extend down to patients with reproductive capacity The age of onset for Parkinson's disease is between 20 and 80, onset between 20 and 40 is referred to as "young-onset Parkinson's disease [24] In a review of 35 pregnancies in patients with Parkinson's disease, there was a suggestion that pregnancy may worsen "off" time in Parkinson's patients [25] In another case, a 33 year old woman had a Hoehn and Yahr rating of 2 with off periods of 13-23% during pregnancy and 16-45% during the first four months post partum, suggesting that pregnancy may increase the potential for apomorphine use [26] For patients with impairment of sexual function, new therapies (viagra[27]) are available for treatment of sexual dysfunction Apomorphine also induces erections and can enable patients to resume sexual relations [28] In summary, the patient population who may use apomorphine includes patients with reproductive potential

The sponsor suggests that a previously published Segment I study (Youssef et al 1999) could be used to fulfil the reproductive toxicity requirement [29] However, the full study report would be needed to permit a full evaluation of the report The paper did not present the data on sperm parameters or testes weight in the 13 week toxicity study In addition, there are suggestive data that reproductive capacity was decreased at the high dose (decreased fertility index [79 2% versus 95 8% in control] and a slight decrease in sperm motility with increase standard error (deviation? Paper does not specify) was observed [96 6% $\pm$ 9 2 versus 98 9 $\pm$ 0 8 in controls]) A more detailed review would be needed to evaluate the significance of this study In addition, this study does not fulfill the requirements for segment II or segment III studies The sponsor also refers to a European Agency for the Evaluation of Medicinal Products report on this study However, the study appears to be the property of Abbot Laboratories Limited and is not in the Public Domain The sponsor would need a Letter of Authorization, which they do not have, to use this study

Finally, the sponsor suggests that the negative result in the in vivo mouse micronucleus and rat unscheduled DNA synthesis test offers reassurance that apomorphine is not genotoxic in vivo and so is unlikely to cause reproductive toxicity This is a fallacious argument It assumes that in order for a drug to be teratogenic it must be positive in the in vivo genotoxicity assays But there are multiple pathways by which substances may affect reproductive performance without affecting genetic material For example, two known human teratogens, thalidomide and

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[24] Fahn, S and S Przedborski (2000) Parkinsonism IN *Merritt s Neruology* (10th Ed, LP Rowland, ed), pages 679-693

[25] Hagell, P, P odin and E Vinge (1998) Pregnancy in Parkinson's disease a review of the literature and a case report Mov Disord 13(1) 34-38

[26] Shulman, LM, Minagar, A and WJ Weiner (2000) The effect of pregnancy in Parkinson's disease Move Disord 15(1) 132-135

[27] Raffaele, R I Vecchio, B Giammusso G Morgia MB Brunetto and L Rampello (2002) Efficacy and safety of fixed-dose oral sildenafil in the treatment of sexual dysfunction in depressed patients with idiopathic Parkinson s disease Eur Urol 41 382-386

[28] O'Sullivan, JD and AJ Hughes (1998) Apomorphine-induced penile erections in Parkinson's disease Move Disord 13(3) 536-539

[29] Youssef, AF FL Fort B Ronsen, R Schroeder, K Williams and C Auletta (1999) Evaluation of apomorphine HCl effects on reproductive endpoints, studies in male rats and dogs Toxicol Sci 51 273-279

isotretinoin, are negative not only in the in vivo mouse micronucleus test but also in in vitro genotoxicity assays (see drug labels) A negative in vivo genotoxicity test is inadequate to discount the potential for reproductive toxicity

In conclusion, the sponsor's arguments for a waiver of the reproductive toxicity tests are inadequate to excuse them from conducting the tests Patients have a right to know whether a drug that they are taking has potential effects on the reproductive system The sponsor should be required to perform these studies prior to approval

Labeling review

The following changes are recommended in the label (label as of 4/7/2003)

DISCRIPTION First sentence delete the word —

CLINICAL PHARMACOLOGY Mechanism of Action Replace the first two paragraphs with the following

[ ]

[ ]

The precise mechanism of action of TRADENAME as a treatment for Parkinson's disease is unknown, although it is believed to be due to stimulation of post-synaptic dopamine D2-type receptors within the caudate-putamen in the brain

In particular, apomorphine attenuates the motor deficits induced by lesioning the ascending nigrostriatal dopaminergic pathway with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in primates

This will make the label consistent with other dopaminergic agonist labels (eg Requip)

PRECAUTIONS Add the following

Retinal pathology in albino rats Retinal degeneration has been observed in albino rats treated with dopamine agonists for prolonged periods (generally during 2-year carcinogenicity studies) This lesion has also been observed when albino rats were exposed for shorter periods under higher intensity light exposures Similar changes have not been observed in 2-year carcinogenicity studies in albino mice or in rats or monkeys treated for 1 year Apomorphine has not been tested in carcinogenicity studies, but based on its mechanism of action it is expected to cause similar toxicity

The significance of this effect in humans has not been established, but cannot be disregarded because disruption of a mechanism that is universally present in vertebrates (e.g., disk shedding) may be involved.

PRECAUTIONS: Information For Patients: Change the 5th through 7th paragraphs as follows:

Because apomorphine has not been evaluated for effects on reproduction, patients should be advised to notify their physicians if they become pregnant or intend to become pregnant during therapy (see PRECAUTIONS: Pregnancy).

Because of the possibility that apomorphine may be excreted in breast milk, patients should be advised to notify their physicians if they intend to breast-feed.

Rare cases of abuse (use of apomorphine significantly in excess of prescribed frequency) have been reported. Apomorphine abuse is associated with inappropriate sexual behavior.

PRECAUTIONS: Drug Interactions: *Dopamine Antagonists*: add the following sentence at the end of the paragraph:

Patients with major psychotic disorders, treated with neuroleptics, should only be treated with dopamine agonists if the potential benefits outweigh the risks.

PRECAUTIONS: Carcinogenesis, Mutagenesis, Impairment of Fertility: Change this sections as follows:

[redacted]

Pregnancy

Pregnancy Category C: reproduction studies have not been conducted with apomorphine.

[redacted]

Studies Delete this section

APPEARS THIS WAY
ON ORIGINAL

RECOMMENDATIONS

The purpose of preclinical studies is to provide information which will supplement the clinical data in assessing the safety of a drug product The preclinical studies provide data which can not be obtained in clinical studies but are of potential concerns to patients These studies include carcinogenicity studies, reproductive toxicity studies and genotoxicity studies Preclinical studies also assist in the evaluation of the clinical studies by providing data on potential target organs

The available preclinical data are inadequate to support the approval of this drug product The sponsor has not conducted the reproductive toxicity studies required for the approval of drugs for Parkinson's disease The sponsor's request for a waiver of these studies does not present a compelling argument **It is recommended that the sponsor conduct and submit the reproductive toxicity studies specified in ICH guidelines prior to drug approval Alternatively, the sponsor may choose to obtain a Letter of Authorization from a sponsor that has already conducted these studies**

Another serious concern is the lack of carcinogenicity studies The sponsor has requested a waiver from conducting carcinogenicity studies However, the sponsor has not presented a compelling argument for a waiver from conducting carcinogenicity studies Given the strong genotoxic responses observed in the in vitro genotoxicity tests, this reviewer considers it imperative that the carcinogenic potential of apomorphine be addressed prior to approval **It is recommended that carcinogenicity studies in mice and rats be conducted prior to approval of this product**

The sponsor has set specifications for a pair of degradation products which are above the threshold of qualification **If the sponsor is unable to lower the specification for these products, it is recommended that the sponsor conduct a qualification study of the degradation product prior to approval of the drug product**

Changes to the label outlined above should be implemented prior to drug approval

There are additional concerns which the sponsor may address during the Phase IV period The in vivo micronucleus test should be repeated using a multiple dosing regimen which would more closely resemble the suggested clinical use The sponsor also needs to determine the metabolism of apomorphine by conducting mass balance studies in mice, rats and monkeys

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