CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-421

PHARMACOLOGY REVIEW(S)

MEMORANDUM

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

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

Date: August 20, 2009

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 22-421 (Mirapex® ER™), Serial 000 received 10/24/08

Mirapex (pramipexole) is a non-ergot dopamine (D_2 subfamily) receptor agonist. The NDA (20-667) for Mirapex IR for treatment of the signs and symptoms of idiopathic Parkinson's disease was approved on July 1, 1997. To support approval of Mirapex ER for treatment of the signs and symptoms of early Parkinson's disease, the sponsor (Boehringer Ingelheim Pharmaceuticals) has referenced the nonclinical data submitted to NDA 20-667 held by this sponsor for Mirapex IR. In addition, the sponsor has provided a number of studies to address the presence of three impurities (Products Z and V, CD 10503) in the drug product. These include studies conducted to fulfill post-approval commitments stated in the Agency's approval letter (7/20/2007) for a 0.75 mg tablet under NDA 20-667/S-016:

1. Description of Commitment:
   An in vitro chromosomal aberration assay in mammalian cells to assess the potential genotoxicity of Products Z and V.

Protocol Submission:	July 20, 2007
Study Start:	Beginning of November 2007
Final Report Submission:	End of January 2008

2. Description of Commitment
   An in vitro mouse lymphoma tk assay (with colony sizing) to assess the potential genotoxicity of Products Z and V.

Protocol Submission:	July 20, 2007
Study Start:	Beginning of November 2007
Final Report Submission:	End of January 2008

3. Description of Commitment
   An in vivo micronucleus assay to assess the potential genotoxicity of Products Z and V.

Protocol Submission:	July 20, 2007
Study Start:	Beginning of November 2007
Final Report Submission:	End of January 2008

The nonclinical studies have been reviewed by Terry S. Peters, D.V.M. (Pharmacology/Toxicology Review and Evaluation, NDA 22-421, 8/19/09). Based on her review, Dr. Peters has concluded that the sponsor's specification limits for Product Z, Product V, and CD 10503 are acceptable. The studies of Products Z and V (but not of CD 10503) were also reviewed by Anita Bigger, Ph.D., a CDER genetic toxicology expert; Dr. Bigger's consult (5/26/09) is provided within Dr. Peters' review. Based on her review, Dr. Bigger has concluded that "…the sponsor should be encouraged to lower the amounts of Product Z and Product V as low as possible."

- The following summarizes the genetic toxicology study results for the three impurities:

Degradants Products Z and V each have a specification limit under "unspecified degradation products" of ≤0.4%. This specification limit is below the level of qualification (cf. *Guidance for Industry Q3B(R2) Impurities in New Drug Products July 2006 ICH Revision 2*). However, preliminary studies conducted by the sponsor identified Products Z + V as having genotoxic potential. Therefore, the sponsor conducted a number of genotoxicity assays assessing the genotoxic potential of the mixture or of purified Product Z or Product V, each alone.

Product Z has previously been shown to be negative in the *in vitro* Ames assay. Both Products Z and V were negative in the *in vivo* micronucleus assay (males only) at single oral doses of 0, 1, 10, and 20 mg/kg (Product Z) or 0, 1, 20, and 30 mg/kg (Product V). The high doses used in the *in vivo* assays were adequate since Product Z was lethal in mice at single oral doses of ≥30 mg/kg and Product V was lethal in mice at single oral doses of 50 and 100 mg/kg. Products Z and V were, however, positive in the *in vitro* mouse lymphoma tk assay and the *in vitro* chromosomal aberration assay in human lymphocytes.

In Study 07b134, a "mixture" containing Products Z (7.9%) and V (7.6%) was tested in the *in vitro* mouse lymphoma tk assay. Since the mixture also contained pyrocatechol (catechol) at 1.4%, catechol was tested separately. The results of this assay are summarized in the following table (COMPD = compound; COND = assay conditions; CONC = concentration [µg/mL]; MF = mutant frequency; SMALL/LARGE = colony sizes):

COMPD	COND	CONC	CYTOTOXICITY		MF		
			RS%	RTG%	SMALL	LARGE	TOTAL
mixture	4-hr, -S9	0	100	100	49	79	133
		10	98	91	42	74	121
		100	48	51	134	159	331
		150	24	26	152	169	360
		200	17	17	201	193	441
		225	16	13	209	247	536
		250	18	14	166	196	424
catechol	4-hr, -S9	0	100	100	48	132	195
		0.1	101	96	51	135	193
		0.5	99	90	69	153	236
		1	93	86	64	15	230
		2.5	83	81	107	178	327
		5	41	42	146	249	447
mixture	24-hr, -S9	0	100	100	26	61	90
		1	124	109	24	66	94
		10	111	87	45	80	128
		100	14	7	390	422	1022
		150	2	1	--	--	--
	4-hr, +S9	0	100	100	30	97	133
		10	110	104	29	110	146
		50	89	80	54	157	219
		100	79	67	103	254	400
		250	36	17	258	415	821
		300	29	15	260	394	807
		500	21	10	299	595	1169
catechol	4-hr, +S9	0	100	100	38	142	194
		0.1	103	97	39	117	162
		0.5	104	95	50	137	200
		1	95	76	52	175	237
		2.5	94	78	91	166	284
		5	58	53	134	260	462

An increase in mutant frequency was observed with the mixture and catechol, both in the absence and presence of metabolic activation. Due to the positive response with catechol, the sponsor investigated the possibility of formation of catechol during the incubation period. The data indicated that during the 4-hr treatment period, concentrations of Products Z and V declined, while those of catechol increased. At the beginning of treatment, concentrations were 7.5, 6.7, and 1.7% w/w, respectively, whereas at the end of 4 hrs, concentrations were 6.1, 5.5, and 2.6% w/w, respectively. The sponsor suggested that catechol may be responsible for the increases in MF, but concluded that "…it cannot be excluded that products Z and V contributed to the mutant frequency…" However, considering the mixture tested, it is not possible to determine based on these data alone what compound or compounds are responsible for the positive findings in this assay.

In Study 07b127, a mixture containing Products Z and V (7.9 and 7.6%, respectively) was tested in the *in vitro* chromosomal aberration assay. Pyrocatechol (catechol) was added as a reference since it was present in the mixture at a level of 1.4%. The total (and %) aberrant cells represents data excluding gaps.

COMPD	COND	CONC µg/mL	MI (%)	Type and Incidence of Aberrations							Aberrant cells	
				g	ctb	csb	ace	td	cte	cse	total	%
1	4-hr; -S9	0	100	2	0	0	0	0	0	0	0	0
		30	90	5	2	0	1	0	0	0	3	1.5
		100	86	3	0	0	1	0	0	0	1	0.5
		300#	65	2	0	0	2	0	0	0	2	1.0
		1000#	18	--	--	--	--	--	--	--	--	--
mixture	24-hr; -S9, 72-hr harvest	0	100	4	0	0	1	0	0	0	1	0.5
		10	86	2	2	0	2	0	0	0	4	2.0
		30	72	3	4	0	1	0	0	0	5	2.5
		100	62	4	6	0	1	0	1	0	8	4.0
		300#	18	18	--	--	--	--	--	--	--	--
	24-hr; -S9, 96 hr harvest	0	100	3	0	0	2	0	0	0	2	1.0
		100	67	4	2	0	2	0	0	0	4	2.0
		300#	25	5	4	0	6	0	2	0	12	6.4
	4-hr; +S9	0	100	1	0	0	1	0	0	0	1	0.5
		30	91	1	3	0	0	0	0	0	3	1.5
		100	95	1	1	0	0	0	1	0	2	1.0
		300#	97	5	1	0	0	0	0	0	1	0.5
		1000#	32	1	0	0	2	0	4	0	6	5.9
catechol	4-hr; -S9	0	100	4	2	0	0	0	0	0	2	1.0
		5	97	2	2	0	3	0	0	0	5	2.5
	4-hr; +S9	0	100	3	1	0	1	0	1	0	3	1.5
		1	125	4	6	0	4	0	2	0	12	6.0
		2.5	113	3	6	0	5	0	5	0	16	8.0

#black colored cultures; -- = not tested

In this assay, both the mixture and catechol induced structural aberrations in the absence of metabolic activation after 24 hrs of treatment. The mixture did not clearly induce structural aberrations in the presence of metabolic activation (although the concentrations tested were inadequate, as noted by Dr. Bigger), whereas catechol did. Dr. Bigger concluded that the results of this assay suggested that catechol was responsible for the positive response of the mixture.

To further investigate the positive responses obtained with the mixture, the sponsor conducted *in vitro* mouse lymphoma tk assays on purified Products Z (Study 08b082) and V (Study 08b113) each tested alone. The sponsor demonstrated that both Products Z and V degraded during incubation in medium, resulting in increasing levels of catechol (sponsor's figures follow).

The sponsor estimated concentrations of catechol of 0, 1.8, 3.8, and 5.7 µg/mL at 0, 1, 2, and 3 hrs of incubation, respectively, with Product Z (100 µg/mL), and concentrations of 0, 2.8, 5.8, and 8.9 µg/mL at 0, 1, 2, and 3 hrs of incubation, respectively, with Product V (100 µg/mL). The results of these studies are summarized in the following table (COND = assay conditions; CONC = concentration [µg/mL]; MF = mutant frequency; SMALL/LARGE = colony sizes):

COND	CONC	CYTOTOXICITY		MF		
		RS%	RTG%	SMALL	LARGE	TOTAL
Product Z						
4-hr, -S9	0	100	100	47	93	149
	10	85	107	49	73	128
	30	78	96	68	137	222
	100	37	51	119	179	359
	200	31	27	164	220	475
	300	22	22	131	231	425
4-hr, +S9	0	100	100	30	91	125
	10	92	98	25	95	124
	30	69	71	44	125	180
	100	50	52	102	210	371
	200	36	29	128	234	426
	300	28	24	130	234	439
Product V						
4-hr, -S9	0	100	100	34	64	102
	10	84	105	39	69	114
	30	94	110	54	97	166
	100	48	62	101	218	366
	200	33	32	122	231	415
	400	19	20	137	237	440
	500	16	21	118	194	358
4-hr, +S9	0	100	100	25	69	97
	10	104	99	27	73	104
	30	102	86	34	71	110
	100	69	59	101	143	272
	200	43	34	128	186	375
	400	30	29	104	150	291
	500	35	26	88	168	293

The data from this study indicate that purified Products Z and V are mutagenic and clastogenic in the absence and presence of metabolic activation. The sponsor and Dr. Bigger both note that the positive responses with the purified impurities (at 100 µg/mL) were of similar magnitude as those induced by catechol in Study 07b134. However, the one notable difference between the mixture and the purified impurities was that, with the mixture, the positive response in the presence of metabolic activation was >2-fold greater than that in the absence of metabolic activation. This effect of metabolic activation was not observed with catechol or purified Products Z and V. This would suggest that something in the mixture other than catechol was induced by metabolic activation and that, therefore, the positive responses obtained with the mixture could not be attributed entirely to catechol (or Products Z or V). According to the CoA provided in Study 07b134, the mixture contained the following: Product Z (7.9%), Product V (7.6%), (b) (4) Due to the possibility that (b) (4) (b) (4) is genotoxic, the sponsor was asked to specify whether or not either of these

impurities is present in Mirapex ER. In an 8/17/09 submission, the sponsor noted that (b) (4) were not detected at levels above the reporting threshold (0.1%) "even after 30 months of storage at 40°C/75% r.h. in the commercial packaging configuration.." According to the chemistry team, it is very unlikely that these impurities, being photodegradants, would be formed upon storage under the conditions specified for the to-be-marketed product.

CD 10503 is a new degradation product, discovered during stability testing of Mirapex ER. According to the sponsor, CD10503 is formed (b) (4) The excipient, hydroxypropyl methylcellulose, (b) (4). According to the sponsor, "Chemical analysis…showed that the meta-stable CD 10503 decomposes in aqueous solution into (b) (4)

Figure 3.2: 1 Equilibrium equation of CD 10503 / (b) (4)

Since the specification limit for CD 10503 (1%) is above the qualification threshold, the sponsor conducted a 13-week oral toxicity study (Study 07B113) of pramipexole (25 mg/kg) spiked with CD 10503 (0, 0, 0.5, 1.0, 2.5 mg/kg) and pramipexole alone. This study was reviewed by Dr. Peters and found to demonstrate no toxicity unique to CD 10503. CD 10503 was, however, positive for mutagenicity in the sponsor's SAR/DEREK analysis; this is a strong signal considering the fact that DEREK has been reported to be a relatively insensitive computational database for predicting Ames positivity. CD 10503 is present at a level of (b) (4) in the drug product; however, at the specification limit of ≤1%, the maximum intake for this degradant would be (b) (4). This exceeds the maximum allowable limit (1.5 µg/day) for a genotoxic impurity in a drug product intended for chronic use. Therefore, the sponsor conducted *in vitro* and *in vivo* genetic toxicology studies of CD 10503.

In vitro Ames assays for CD 10503: CD 10503 was clearly positive in 3 tester strains in the Ames assay, +/- S9. The data (revertants/plate) from Study 07B016 are summarized in the following table:

S9	CD10503 CONC	TESTER STRAIN				
		TA1535	TA1537	TA98	TA100	TA102
-	0	12	5	45	104	404
	10	11	5	54	122	457
	25	13	5	65	140	557
	50	14	8	98	130	617
	100	15	10	114	246	1015
	200	20*	12*	52*	330	1152
+	0	11	6	38	112	461
	10	13	11	55	119	503
	25	14	8	63	141	537
	50	15	11	79	163	577
	100	16	10	84	242	857
	200	22*	8*	46*	330	1042

*sponsor notes toxicity

As a result of these findings, the sponsor conducted mechanistic studies to determine the mechanism(s) responsible for the Ames-positive signals. All mechanistic *in vitro* Ames assays were conducted in the absence of metabolic activation. The results of those studies (revertants/plate) are summarized in the following tables (FA = formaldehyde; FDH = formaldehyde dehydrogenase; SEM = semicarbazide; CONC in µg/plate; -- = not tested):

CD10503 CONC	TA 98		TA 100				TA 102			
	CD10503	CD10503 + SEM#	CD10503	CD10503 + FDH		CD10503 + SEM#	CD10503	CD10503 + FDH	CD10503	CD10503 + SEM#
				0.5 U	2.5 U			1.0 U		
0	26	27	131	143	134	132	386	391	408	407
10	29	35	138	135	139	155	388	398	431	443
25	41	31	148	135	146	154	418	430	493	466
50	69	41	183	152	159	155	517	582	598	482
100	94	84	305	185	172	163	611	865	778	534
200	65*	60*	387	323	215	179	742	1417	922	635

*sponsor notes toxicity, #SEM concentrations of 6.2, 15.6, 31.2, 62.5, 125 µg/plate at CD 10503 concentrations of 10, 25, 50, 100, 200 µg/plate, respectively.

FA CONC	TA100			TA102	
	FA	FA + FDH		FA	FA + FDH
		0.5 U	2.5 U		1.0 U
0	131	143	143	386	391
2	160	145	145	425	394
5	182	149	139	502	469
10	256	155	147	653	572
25	485	252	193	1070	1222
50	258*	382	242	1040	1630

*sponsor notes toxicity

CONC		TA98			TA100			TA102		
FA	SEM	FA	FA + SEM	SEM	FA	FA + SEM	SEM	FA	FA + SEM	SEM
0	0	26	27	26	131	132	132	408	407	408
2	5	47	35	--	160	137	--	449	412	--
--	10	--	--	45	--	--	141	--	--	388
5	25	63	34	--	182	157	--	514	418	--
10	50	89	51	49	256	147	--	589	449	442
--	100	-	--	33*	--	--	149	--		440
25	125	96	66	--	485	189	--	790	477	--
50	250	12*	56	14*	258*	151*	--	1043	449	381
--	500	--	--	12*	--	--	--	--	--	315*
--	1000	--	--	--	--	--	216*	--	--	--

*sponsor notes toxicity

The sponsor investigated the degradation kinetics of CD 10503; study results indicated that, in an aqueous medium (80% PBS/20% DMSO), CD 10503 degraded to pramipexole and FA, with equilibrium being achieved by 30 minutes. The sponsor estimated that FA was present in cultures at concentrations of 1.6, 3.1, 6.2, 12.5, and 25 µg/plate at CD 10503 concentrations of 10, 25, 50, 100, and 200 µg/plate.

The data for tester strain TA100 indicate that co-incubation of CD 10503 with either FDH (formaldehyde dehydrogenase) or SEM (semicarbazide, a chemical trap) produced a decrease in revertants compared to CD 10503 alone. With TA102, SEM slightly reduced the number of revertants at the highest concentrations of CD 10503 tested, but FDH actually increased the number of revertants at all concentrations tested, with an almost 2-fold increase at the highest concentration of CD 10503 tested. The sponsor has no explanation for why FDH had this effect. With tester strain TA98, SEM produced a small decrease in the number of revertants produced by CD 10503; the effect of FDH was not assessed in this tester strain. Based on these data, CD 10503 was positive in the Ames assay, even in the presence of FDH or SEM, both of which should have reduced the concentration of FA.

In the assays testing FA alone, FA was clearly mutagenic in tester strains TA98, TA100, and TA102, as was CD 10503. When FA was co-incubated with either FDH or SEM, findings similar to those with CD 10503 were obtained. That is, both FDH and SEM reduced the mutation rate of FA with TA100 and SEM reduced the mutation rate of FA with TA102, whereas FDH increased (≈60%) the mutation rate with TA102 compared to FA alone. As for CD 10503, neither FDH nor SEM completely prevented the positive response for FA in the 3 tester strains.

In vitro chromosomal aberration assay in human lymphocytes for CD 10503: the data from Study 07b160 are summarized in the following table (COND = assay conditions; CONC = concentrations; PC = positive control; g = chromatid and chromosome gap; ctb = chromatid break; csb = chromosome break; ace = acentric fragment; td = terminal deletion; cte = chromatid exchange; cse = chromosome exchange; data represent mean of 2 cultures [-gaps], 200 cell scored, except when toxicity decreased scorable cells):

COND	CONC µg/mL	MI (%)	Type and Incidence of Aberrations							Aberrant cells	
			g	ctb	csb	ace	td	cte	cse	total	%
CD 10503											
4-hr; -S9	0	100	5	2	0	4	0	0	0	6	3
	5	98	3	1	0	2	0	0	0	3	1.5
	25	81	3	2	3	7	0	0	0	12	6.0
	50*	61	7	22	8	9	0	42	0	81	40.5
	60*	26	7	23	10	12	0	42	0	87	61.7
	PC	76	10	18	2	27	1	6	0	54	27.0
4-hr; +S9	0	100	7	2	1	3	0	0	0	6	3.0
	5	94	7	1	0	0	0	0	0	1	0.5
	50	80	9	9	6	5	0	21	0	41	20.5
	60*	40	12	20	13	6	0	50	0	89	44.5
	PC	80	15	27	8	11	0	40	0	86	43.0
formaldehyde											
4-hr; -S9	0	100	5	2	0	4	0	0	0	6	3.0
	10*	61	11	20	4	10	0	14	0	48	24.0
	15*	28	8	24	10	7	0	49	0	90	62.5
	PC	76	10	18	2	27	1	6	0	54	27.0
4-hr; +S9	0	100	7	2	1	3	0	0	0	6	3.0
	10*	72	0	8	1	10	0	1	0	20	10.0
	15*	22	9	20	13	4	0	43	0	80	40.0
	PC	80	15	27	8	11	0	40	0	86	43.0

*Sponsor notes toxicity; HC range for aberrant cells: 0-2.5%. PC = adriamycin (-S9), cyclophosphamide (+S9).

The sponsor calculates that (b) (4) of FA is present at 50 µg/mL of CD 10503. Both CD 10503 and FA produced clear positive responses in the absence and presence of metabolic activation. Although the sponsor notes that the pattern of structural aberrations were similar between CD 10503 and FA, it is of note that the PC also produced a similar pattern of structural aberrations. Therefore, the similarity in the pattern of structural aberrations does not provide additional evidence that FA is responsible for the positive response produced by CD 10503.

In vivo micronucleus assay: CD 10503 was negative in the in vivo micronucleus assay (Study 09b129) when tested as pramipexole (25 mg/kg) spiked with CD 10503 (0.5, 1.0, 2.5 mg/kg) at the end of a 13-week oral toxicity study at a maximum dose of 2.5 mg/kg (micronuclei: 0.13% for control, 0.11-0.12% with CD 10503). However, this is not an adequate test of in vivo clastogenicity due to the low doses of CD 10503 tested. It also appears that no positive control was tested.

Conclusions and Recommendations

- **Product Z and Product V**

The data from the studies of a mixture containing Product Z and Product V and of the purified impurities provide evidence, although indirect, that degradation to catechol may be responsible for the mutagenic and clastogenic effects observed. In recent discussions, Dr. Bigger concurred with this conclusion. Although data on catechol in food are not robust, Dr. Bigger points out that the estimated amount of catechol in food is likely to be

>80 fold greater than the amount anticipated in Mirapex ER at the maximum recommended dose of 4.5 mg/day (b) (4)). Dr. Bigger also compared the estimated amount of catechol in the Mirapex ER product to the doses of catechol administered to rats in a 2-year carcinogenicity assay (Health Canada, R08-4391). Based on these data, Dr. Bigger calculated safety margins of ≈20,000 and 85,000 compared to the low-effect dose for hyperplasia of the glandular stomach and the no-effect dose for adenocarcinomas of the glandular stomach, respectively. Taking all these data into consideration, it does not appear that the specification limits for Product Z and Product V represent a safety concern.

- **CD 10503**

Overall, the data from the studies of CD 10503 provide evidence, although indirect, that FA (a known mutagen and demonstrated to be so in these studies) may be responsible for the mutagenic effect of CD 10503. The mechanistic data conducted in the Ames tester strains might have been more convincing if the sponsor had tested CD 10503 in the presence of higher concentrations of FDH. However, based on the available data and maximum amount of FA anticipated to be present in the Mirapex ER formulation (b) (4) based on the assumption that there will be equimolar formation of FA, a specification limit for CD 10503 of (b) (4) and a maximum human dose of 4.5 mg/day), there appears to be no safety concern. The US/EPA has set an Acceptable Daily Intake for FA of 0.2 mg/kg (12 mg/day for a 60 kg human), an amount (b) (4) times the anticipated maximum daily dose of FA with Mirapex ER.

Therefore, from a pharmacology/toxicology standpoint, there is no objection to approval of the NDA.

Recommended labeling

(b) (4)

2 Page(s) of Draft Labeling has been withheld in full immediately following this page as B4 (CCI/TS)

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22421	ORIG 1	BOEHRINGER INGELHEIM PHARMACEUTICALS INC	PRAMIPEXOLE DIHYDROCHLORIDE

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/s/

LOIS M FREED
08/20/2009

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-421**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**10/24/2008**
PRODUCT:	**Mirapex® ER™ (pramipexole dihydrochloride)**
INTENDED CLINICAL POPULATION:	Patients with idiopathic Parkinson's disease
SPONSOR:	**Boehringer Ingelheim Pharmaceuticals Inc.**
DOCUMENTS REVIEWED:	**Electronic submission**
REVIEW DIVISION:	**Division of Neurology Products**
PHARM/TOX REVIEWER:	**Terry S. Peters, D.V.M.**
PHARM/TOX SUPERVISOR:	**Lois M. Freed, Ph.D.**
DIVISION DIRECTOR:	**Russell Katz, M.D.**
PROJECT MANAGER:	**Beverly Connor**

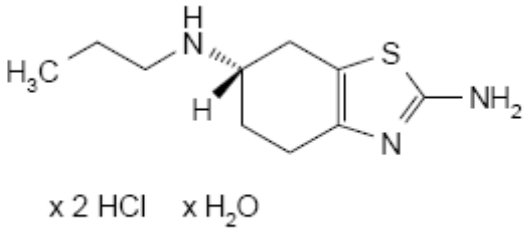
Date of review submission to Division File System (DFS): 8/12/09

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-421
Review number: 1
Sequence number/date/type of submission: N000; 10/24/08; original NDA
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT
Manufacturer for drug substance: Boehringer Ingelheim Pharmaceuticals GmbH, Germany
Reviewer name: Terry S. Peters, D.V.M.
Division name: Neurology Products
Drug:

Trade name: Mirapex® ER™
Generic name: Pramipexole dihydrochloride extended release
Code name: Not specified
Chemical name: (S)-2-amino-4,5,6,7- tetrahydro-6-(propylamino) benzothiazole
Molecular formula/molecular weight: $C_{10}H_{17}N_3S$; 211.33 Daltons
Structure:



Relevant INDs/NDAs/DMFs: Mirapex® (pramipexole dihydrochloride) NDA 20-667, originally approved in 1997.

Drug class: Amino-benzothiozole dopamine agonist with selectivity for the D_2 subfamily with higher affinity for D_3 receptor subtypes than D_2 or D_4 subtypes

Intended clinical population: Patients with Parkinson's disease

Clinical formulation: Pramipexole tablets with 0.375, 0.75, 1.5, 3.0 and 4.5 mg active ingredient. The inactive ingredients include: hypromellose (b) (4), corn starch, carbomer (b) (4), colloidal silicon dioxide and magnesium stearate.

Route of administration: Oral

Previous clinical experience: There is an extensive history of the use of pramipexole and the sponsor is relying on the safety and efficacy information from NDA 20-667 to support this formulation, as well as 2 randomized, double-blind clinical trials in patients with the new formulation.

Disclaimer: Tabular and graphical information are constructed by the sponsor unless cited otherwise.

Studies reviewed within this submission:

1) **Pramipexole ER (SND 919 CL2 Y and impurity CD 10503): 13-Week Oral (Gavage) Toxicity Study in Rats**

2) **CD 10503 (degradation product of pramipexole ER): Mutagenicity study using the S. typhimurium/mammalian microsome assay (Ames test)**

3) **CD 10503 (degradation product of pramipexole ER): Mechanistic follow-up mutagenicity study using the S. typhimurium/mammalian microsome assay (non-GLP)**

4) **Product Z (degradation product of pramipexole): Mutagenicity study for chromosomal aberrations in human lymphocytes in vitro**

5) **Product Z (degradation product of pramipexole): Mutagenicity study using the mouse lymphoma (L5178Y) assay**

6) **Pramipexole Product Z (degradation product): Mutagenicity study using the mouse lymphoma (L5178Y) assay**

7) **CD 10503 (degradation product of pramipexole): Mutagenicity study from chromosomal aberrations in human lymphocytes in vitro**

8) **Pramipexole Product Z (degradation product): Mutagenicity study using the mouse lymphoma (L5178Y) assay**

9) **Pramipexole Product V (degradation product): Mutagenicity study using micronucleus analysis of rat bone marrow after oral treatment**

10) **Pramipexole Product Z (degradation product): Mutagenicity study using micronucleus analysis of rat bone marrow after oral treatment**

11) **Pramipexole Product V (degradation product): Mutagenicity study using the mouse lymphoma (L5178Y) assay**

2.6.2 PHARMACOLOGY

No new information

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

No new information

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

Study title: **Pramipexole ER (SND 919 CL2 Y and impurity CD 10503): 13-Week Oral (Gavage) Toxicity Study in Rats**

Key study findings: There were no new toxicities discovered with co-administration of pramipexole dihydrochloride spiked with CD 10503 (a degradation product) when given to rats by oral gavage for 13 weeks. Adverse clinical signs (initial hypoactivity followed by hyperactivity) were noted throughout the dosing period but resolved during the recovery period. Histologic lesions (ovarian hyperplasia with increased numbers of corpora lutea; gastric mucosal erosions and hemorrhages) were found in approximately equal incidence and severity in animals treated with pramipexole with or without spiked CD 10503. The lesions were essentially resolved by the end of the 6 week recovery period. No NOAEL for this study can be set on the basis of the adverse clinical signs and histologic findings, but it does not appear that the co-administration of CD 10503 elicited additional toxicity. No increases in polychromatic erythrocytes were found after evaluation of bone marrow cells from treated male rats.

Study no.: 07B113 (toxicity portion) and 07B129 (micronucleus portion)
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany and (b) (4)
Date of study initiation: 9/18/07
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: Dihydrochloride salt of SND 919 (pramipexole), Lot 1021848 at 99.8% purity and the degradation product CD 10503, Lot PR4PAC02486A1 at 53.3% potency (conversion factor: 1.87)

Methods

Doses: SND 919 CL2Y (pramipexole) at 0 (Group 1; vehicle control), 35.7 (equal to 25 mg/kg/d SND 919 BS) mg/kg/d for remaining groups with varying amounts of CD 10503 (Group 2: 0; Group 3: 0.5 mg/kg/d; Group 4: 1.0 mg/kg/d; Group 5: 2.5 mg/kg/d). Doses were based on Study U87-1039 (13 week study in Wistar rats) at doses of 0.5, 4 and 25 mg/kg/d of pramipexole.

Group	Daily dose SND 919 BS [mg/kg]	Daily dose CD10503 [mg/kg]
1	0	0
2	25	0
3	25	0.5
4	25	1.0
5	25	2.5

Species/strain: Crl:WI(Han) SPF rats

Number/sex/group or time point (main study): 10

Route, formulation, volume, and infusion rate: Oral gavage in "acidulated demineralized water" at 10 mL/kg

Satellite groups used for toxicokinetics or recovery: 10/sex from control and Group 5 maintained for an additional 6 weeks without dosing.

Age: 9-10 weeks of age at study initiation

Weight: 156.3- 289.4 gms

Sampling times: Four animals/sex were sampled for SND 919 BS plasma levels on Days 1 and 91 at 1, 3, 8 and 24 hrs post-dosing.

Unique study design or methodology: The animals were group-housed. At main study necropsy, samples were taken for evaluation of micronucleus induction.

Observation and Times:

Clinical signs: At least 2x/day
Body weights: Twice weekly during the dosing period, weekly thereafter
Food consumption: As for body weight
Ophthalmoscopy: Prior to study initiation and Days 87 and 129
EKG: Not performed
Hematology: Days 3, 92 and 134 via retrobulbar venous puncture under anesthesia
Clinical chemistry: As for hematology
Urinalysis: Males: Day 85; females: Day 86 and both genders on Day 128 from cage pan samples after bolus water administration of 20 mL/kg approximately 30 min post-daily dosing of test article
Gross pathology: All animals on study
Histopathology: All animals on study

Adequate Battery: yes
Peer review: yes

Results:

Mortality: One designated recovery female from the 25 mg/kg/d pramipexole + 2.5 mg/kg/d CD 10503 (#554) was sacrificed on Day 19 due to adverse signs (hypersensitivity and cold to the touch). Necropsy revealed a gavage accident.

Female #462 (TK group) was humanely sacrificed on Day 1 after the 8 hr sampling and was replaced.

Clinical signs: Treatment-related adverse clinical signs (hypoactivity Days 15-52; hyperactivity Days 53- end of dosing period) were noted in all pramipexole-treated animals. During the hyperactivity phase, a few of the animals had lesions ("encrustations and acrokeratosis") on their forepaws.

Increased water intake (visual assessment only, no measurements taken) was reported for all pramipexole-treated animals beginning on Day 4. This may have been a compensatory mechanism as these animals had glucosuria on urinalysis.

Body weights: All pramipexole-treated animals showed dose-independent, decreased body weights when compared to controls. During the recovery period, normalization occurred with Group 5 males at -9.0% compared to control values. It is difficult to understand the dose independence noted during the study, but there was an obvious treatment-related effect on body weight.

Table 3.2.3: 1 Pramipexole ER (SND 919 CL2Y and impurity CD 10503): 13-week oral (gavage) toxicity study in rats. Mean body weights (g) at start of treatment, treatment and recovery end

Body weight on	Group/gender	1	2	3	4	5
	Dose SND 919 BS [mg/kg]	**0 (Control)**	**25**	**25**	**25**	**25**
	Dose CD 10503 [mg/kg]	**0 (Control)**	**0**	**0.5**	**1.0**	**2.5**
Day 1 [a]	Males	258.01	261.17	255.62	265.73	257.93
	Females	176.23	178.29	178.59	180.89	176.43
Day 91	Males [a]	383.22	324.21 ↓	308.89 ↓	337.98 ↓	328.40 ↓
	Females [b]	237.37	208.90 ↓	210.26 ↓	211.15 ↓	209.15 ↓
Day 133 [c]	Males	419.89	-	-	-	381.93 ↓
	Females	250.99	-	-	-	242.75

↑, ↓: significantly increased, decreased compared with Control; p<= 0.05, many-to-one t-test, two-sided
a: n = 20 per gender in Group 1 and 5, n = 10 in Group 2, 3 and 4
b: n = 20 females in Group 1, n = 19 females in Group 5, n = 10 in Group 2, 3 and 4
c: n = 10 per gender in Group 1 and 5

Food consumption: No significant consistent treatment-related adverse effects on feed consumption were discussed.

Ophthalmoscopy: No treatment-engendered lesions were discovered.

Hematology: Decreased lymphocyte counts were found in males (-24%, -22%, -44% and -36% for the respective treated males compared to controls) on Day 92 but females had increased neutrophil counts (61%, 124%, 76% and 111% for respective treated females compared to controls). No adverse treatment-related effects were noted on red cell parameters. The etiology of these changes is unclear and the findings were not consistent between the genders.

Although no changes in clotting parameters were found, the analyses were done on blood taken from the retro-orbital sinus and the values are not considered valid.

Clinical chemistry: Slightly increased AST values were reported starting on Day 3 and persisting throughout the dosing period. Concomitant ALT values were also increased but not as significantly. The mean increases did not reach toxicologic significance in any group. Although GLDH levels were elevated, the increases were dose independent and not considered toxicologically significant. BUN levels were marginally and dose independently increased.

No intergroup differences were found at the end of the recovery period.

Table 3.4.3.1: 1 Pramipexole ER (SND 919 CL2Y and impurity CD 10503): 13-week oral (gavage) toxicity study in rats. Changes in AST and GLDH activities

Parameter [unit]	Day		Daily dose of pramipexole + CD 10503 [mg/kg]								
			0	25+0		25+0.5		25+1.0		25+2.5	
		Group	1	2		3		4		5	
		Gender	*mean*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*	*mean*	*Δ%*
AST [U/L]	3	M	71.5	157↑	120.2	206↑	188.4	172↑	141.2	194↑	170.9
	3	F	72.6	118↑	63.0	161↑	121.1	116↑	60.2	142↑	96.2
	92	M	62.6	118↑	88.8	133↑	112.4	189↑	202.2	138↑	120.4
	92	F	78.2	114	45.6	188↑	140.7	113	45.0	144↑	84.1
	134	M	63.3	N/A	N/A	N/A	N/A	N/A	N/A	73.7	16.4
	134	F	69.7	N/A	N/A	N/A	N/A	N/A	N/A	70	0.4
GLDH [U/L]	3	M	6.06	17.5↑	189.2	17.9↑	195.8	14.8↑	143.8	14.3↑	136.0
	3	F	6.53	27.8↑	325.4	19.1↑	192.4	14.8	126.4	18.6↑	185.4
	92	M	6.60	5.81	-12.0	7.01	6.2	7.04	6.7	5.67	-14.1
	92	F	9.92	7.15	-27.9	7.09	-28.5	8.56	-13.7	6.98	-29.6
	134	M	7.21	N/A	N/A	N/A	N/A	N/A	N/A	7.38	2.4
	134	F	11.3	N/A	N/A	N/A	N/A	N/A	N/A	5.16	-54.2

M: males; F: females
N/A: not applicable
Δ% percent deviation from Control (calculated from original raw data/rounded)
↑ statistically significant increase compared with Control; p<=0.05, many to one t-test, two sided

Urinalysis: Increased urine volume (≤1.6x controls) and glucosuria were recorded for all treated animals on Day 92 (consistent with the increased water intake).

Gross pathology: Enlarged ovaries were noted in treated females (10/10 for Groups 2 and 3, 9/10 for Groups 4 and 5).

Mucosal "defects" and discoloration were observed in the glandular stomachs of treated males (6/10, 6/10, 6/10 and 1/10 for respective groups) and females (9/10, 10/10, 9/10 and 8/10 for respective groups) at the end of the dosing period. Similar lesions were not found at the end of the recovery period.

Organ weights: Treated females had decreased thymic and pituitary weights. Both absolute and relative ovarian weights were increased in treated females. These increases in ovarian weights persisted throughout the recovery period. When comparing the Group 2 females to controls, thymic weights were decreased 27.4%; when comparing Group 5

females to controls, the thymic decrease was 39%. Ovarian weights were increased 306% for Group 2 females and 343% for Group 5 females when compared to controls.

female		92 [day]							
Parameter		Body wt.	Heart	Lung	Liver	Kidneys	Thymus	Spleen	Ovaries
		[g]	[g]	[g]	[g]	[g]	[g]	[g]	[mg]
Group									
Group 1	n	10	10	10	10	10	10	10	10
0	mv	218.35	0.7685	1.1643	5.1344	1.4433	0.2285	0.4495	94.40
[mg/kg]	sd	13.20	0.0844	0.1056	0.5168	0.1495	0.0366	0.0665	9.09
Group 2	n	10	10	10	10	10	9	10	10
25	mv	184.28	0.8045	1.2101	5.4040	1.4426	0.1659	0.4156	289.67
[mg/kg]	sd	9.18	0.0777	0.0556	0.2982	0.0750	0.0331	0.0447	30.68
	p	* < 0.0001	0.3121	0.2317	0.1707	0.9896	* < 0.0001	0.1459	* < 0.0001
Group 3	n	10	10	10	10	10	10	10	10
25	mv	188.09	0.8226	1.2341	5.4221	1.4183	0.1560	0.4055	312.52
[mg/kg]	sd	11.96	0.0774	0.0779	0.3598	0.0669	0.0274	0.0251	46.92
	p	* < 0.0001	0.1319	0.0711	0.1443	0.6163	* < 0.0001	0.0611	* < 0.0001
Group 4	n	10	10	10	10	10	10	10	10
25	mv	189.61	0.8106	1.2293	5.6607	1.5497	0.1607	0.4346	321.55
[mg/kg]	sd	13.35	0.0636	0.1020	0.5386	0.1342	0.0285	0.0652	39.29
	p	* < 0.0001	0.2389	0.0918	* 0.0093	* 0.0372	* < 0.0001	0.5205	* < 0.0001
Group 5	n	9	9	9	9	9	9	9	9
25	mv	179.00	0.7875	1.1692	5.5173	1.4740	0.1412	0.3930	323.88
[mg/kg]	sd	6.26	0.0896	0.0679	0.3968	0.1033	0.0153	0.0408	52.63
	p	* < 0.0001	0.6016	0.8994	0.0606	0.5493	* < 0.0001	* 0.0205	* < 0.0001

female		92 [day]					
Parameter		Adrenals	Pituitary	Thyroid	Brain	ln. mes.	ln. axill.
		[mg]	[mg]	[mg]	[g]	[mg]	[mg]
Group							
Group 1	n	10	10	10	10	10	10
0	mv	66.48	13.03	15.66	1.9843	201.99	48.70
[mg/kg]	sd	11.61	2.23	2.59	0.0658	56.55	12.33
Group 2	n	10	10	10	10	10	10
25	mv	70.62	9.99	14.59	2.0483	144.55	42.57
[mg/kg]	sd	7.59	1.14	2.00	0.1186	29.24	10.79
	p	0.3593	* 0.0003	0.3782	0.1070	* 0.0035	0.2749
Group 3	n	10	10	10	10	10	10
25	mv	73.24	10.00	12.60	2.0383	142.93	45.81
[mg/kg]	sd	8.05	1.20	2.88	0.0729	36.38	13.53
	p	0.1375	* 0.0003	* 0.0145	0.1724	* 0.0028	0.6048
Group 4	n	10	10	10	10	10	10
25	mv	78.40	11.13	12.76	2.0451	167.55	44.86
[mg/kg]	sd	10.85	1.75	2.90	0.0968	42.87	12.23
	p	* 0.0107	* 0.0169	* 0.0201	0.1251	0.0712	0.4922
Group 5	n	9	9	9	9	9	9
25	mv	74.79	10.33	13.77	2.0426	161.56	39.63
[mg/kg]	sd	11.30	2.00	2.99	0.0671	37.76	13.00
	p	0.0772	* 0.0013	0.1324	0.1517	* 0.0403	0.1186

Histopathology: Adequate Battery: yes
Peer review: yes

As in previous studies with pramipexole, the ovaries of treated females had increased numbers and/or dimensions of corpora lutea. This finding was not detected at the end of the recovery period.

In the stomachs of treated animals, gastric erosions and hemorrhages were described in the glandular mucosa. Most of the lesions were healed by the end of the recovery period.

Incidence summary of microscopic observat., main study animals

Group	1		2		3		4		5		
Group name	*Group 1*		*Group 2*		*Group 3*		*Group 4*		*Group 5*		
Dose level [mg/kg]	0		25		25		25		25		
	m	f	m	f	m	f	m	f	m	f	
Animals in group :	10	10	10	10	10	10	10	10	10	10	
Animals examined :	10	10	10	10	10	10	10	10	10	10	
stomach	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	
-erosion	0	0	3	4	2	7	2	6	2	8	
-foam cell accumulation	0	0	1	1	0	0	0	1	0	0	
-foreign body granuloma	0	0	0	0	0	0	0	0	1	0	
-hemorrhage	0	0	0	4	3	2	1	1	0	0	
-infiltration, inflammatory	0	1	1	2	1	1	2	1	0	2	

Toxicokinetics: Samples were analyzed by HPLC-MS/MS methodology. Test article was not found in any of the control samples. Exposure to SND 919 was independent of degradant concentration and the levels were significantly higher on Day 91 than on Day 1 (Cmax: 6.9x higher than Day 1 and AUC: 4.22x higher than Day 1). No gender-related differences were appreciated. The sponsor suggested that the increase was not due to accumulation but "by a change in the pharmacokinetics with repeated dosing".

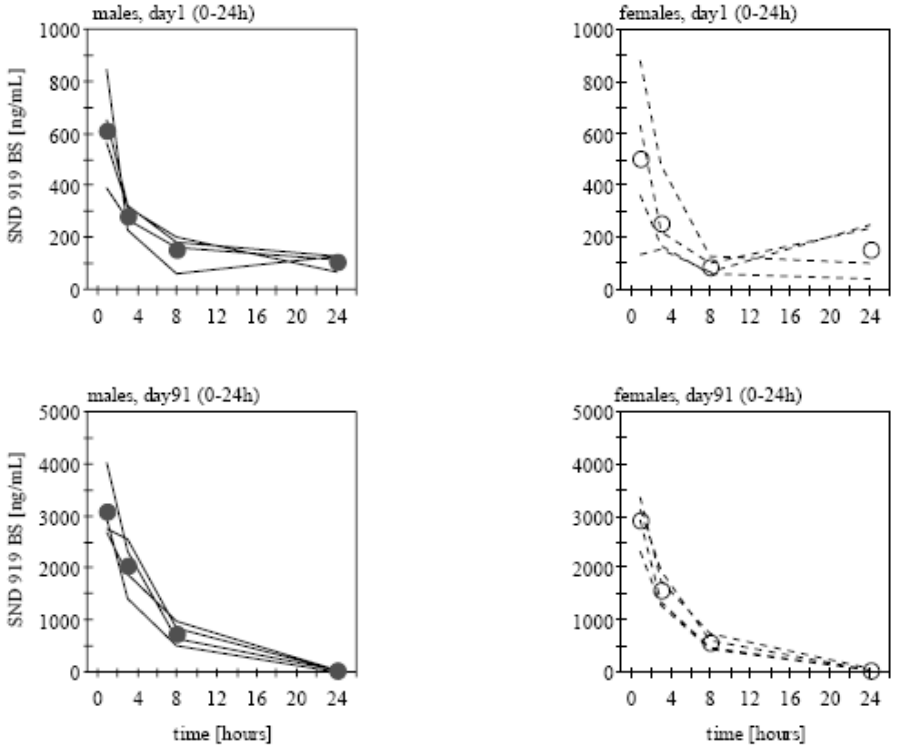
A requested consultation with Dr. Charles Bonapace (OCPB) provided the following:

"It is clear is that the Cmax and AUC0-24 are higher on day 91 because of accumulation. This can occur independent of whether the pharmacokinetic parameters are changing with repeated dosing. What is not clear is whether the pharmacokinetic parameters (e.g., plasma clearance, volume of distribution, and elimination half-life) are changing or not. The sponsor would need to provide more information about these parameters on day 1 and day 91 to demonstrate that they change with repeated dosing. Without more information, it is possible that the accumulation on day 91 (in the Cmax and AUC) is simply due to a long elimination half-life."

Summary Table Mean toxicokinetic parameters after dosing of 25 mg/kg SND 919 BS (dosed as SND 919 CL2Y + CD 10503)

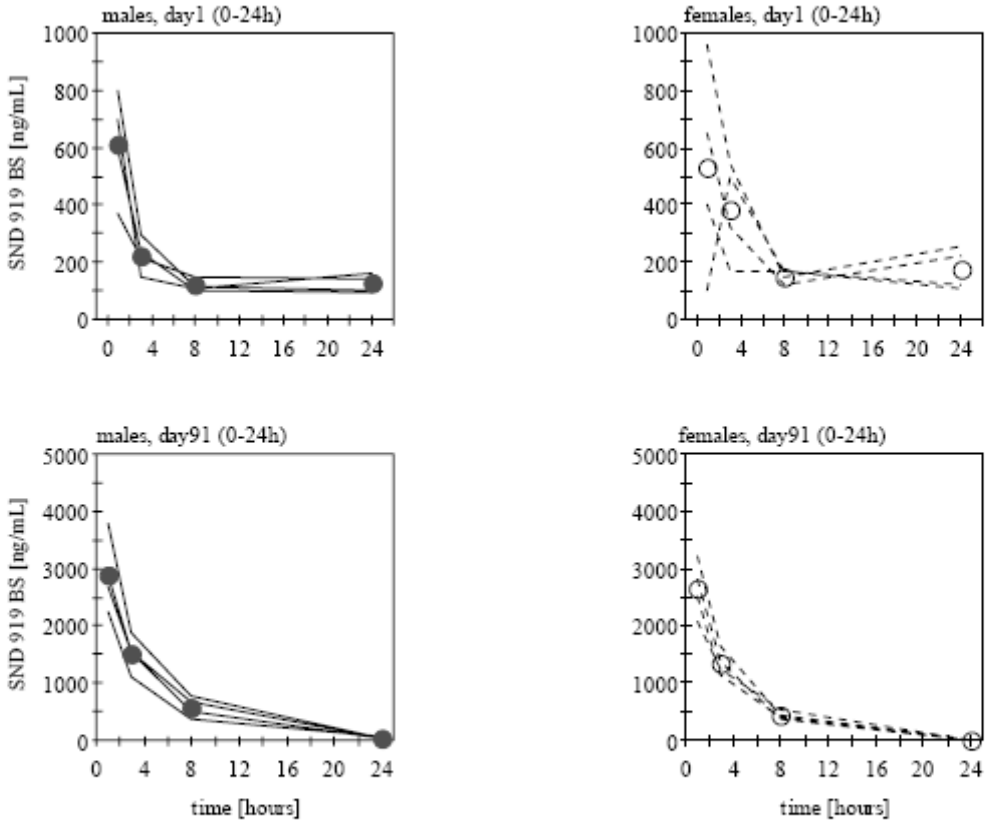
Parameter	Day	Gender	Group 2	Group 3	Group 4	Group 5
	1	m	612	614	527	532
C(max)	1	f	529	626	306	1080
[ng/mL]	91	m	3100	2900	3670	3490
	91	f	2930	2660	2830	3570
	1	m	4370	3940	4330	3990
AUC(0-24h)	1	f	3810	5120	3310	5740
[ng·h/mL]	91	m	19700	16100	21800	18700
	91	f	16100	13500	15400	17500

Group 2



study no.: 07B113
ToxKin code: 07B113_NR1_TK1
raw data ID: 1

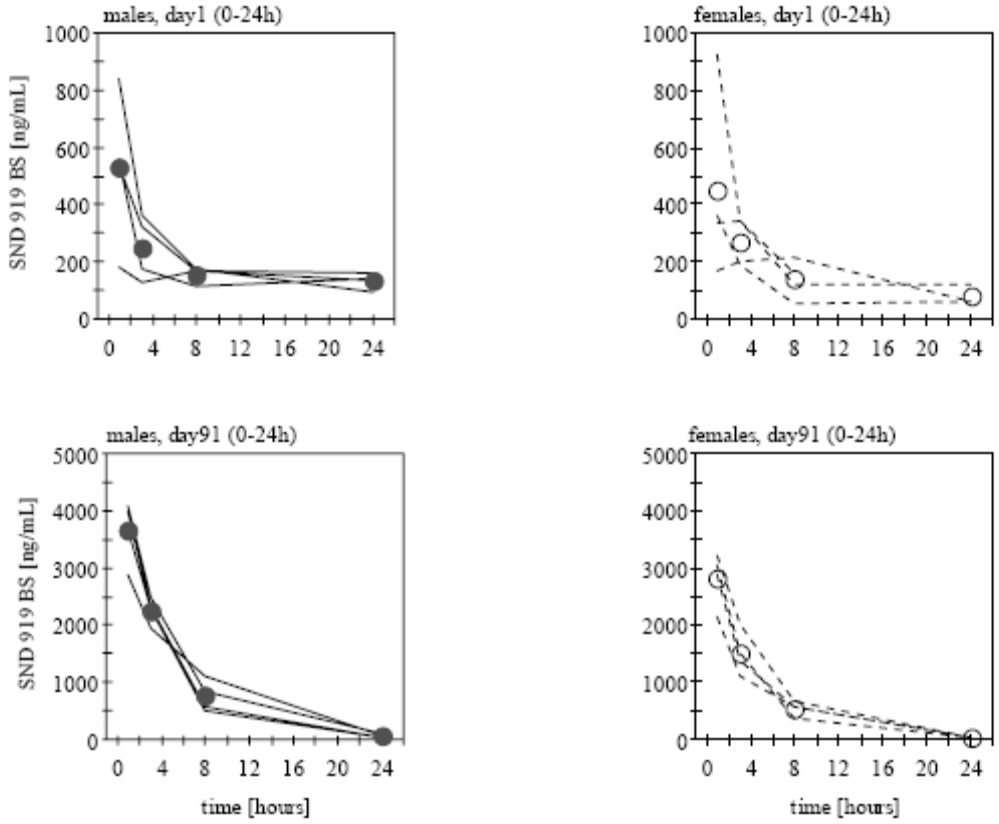
Group 3



study no.: 07B113
ToxKin code: 07B113_NR1_TK1
raw data ID: 2

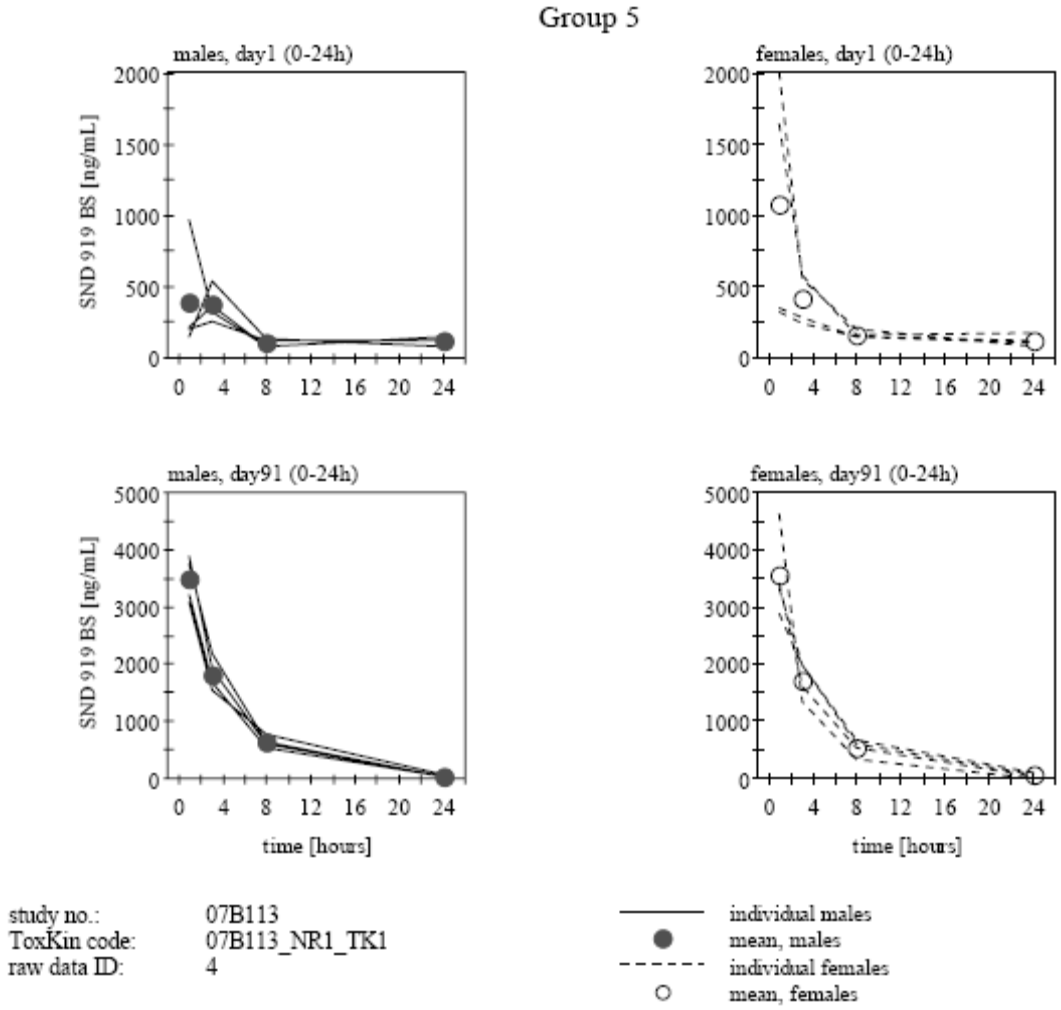
individual males
mean, males
individual females
mean, females

Group 4



study no.: 07B113
ToxKin code: 07B113_NR1_TK1
raw data ID: 3

individual males
mean, males
individual females
mean, females



Other: Evaluation of bone marrow from the males at the end of the main study did not reveal induction of micronuclei in any dose group. Mean control micronucleated PCE values were 0.13% and mean treated groups ranged from 0.11 to 0.12%. A positive control arm was not included in this study.

2.6.6.4 Genetic toxicology

In a previous NDA submission (20-667) for Mirapex®, two degradants, identified as Products Z and V, were identified. The current specifications for these degradants (≤0.4%) does not exceed the ICH reporting threshold (0.1%) in the extended release tablets. The final study reports are included in this submission.

Pramipexole was negative in a battery of genotoxicity assays. In the accelerated chemistry stability testing for the new formulation, an additional degradant (CD 10503) was identified at (b). The specification for this degradant is (b) (4). (b) (4)

The excipient in the ER tablets (hydroxypropyl methylcellulose) (b) (4) . The sponsor's SAR/DEREK analysis showed the degradant to

be a potential mutagen. An Ames assay was performed and the degradant was shown to have mutagenic activity in the study. Mechanistic studies were done to determine the causative agent and the underlying mechanism of action.

Study title: **CD 10503 (degradation product of pramipexole ER): Mutagenicity study using the S. typhimurium/mammalian microsome assay (Ames test)**

Key findings: In strains TA 98, TA 100 and TA 102, a statistically and biologically, dose-dependent increase in mutations was found. Increases of 2-3x the control values were found with and without S9 metabolic activation. Thus, CD 10503 is a bacterial mutagen under the conditions of this assay.

Study no.: 07B016 or U07-1308
Volume and page #: Module 4
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH& Co KG, Biberach an der Riss, Germany
Date of study initiation: 2/22/07
GLP compliance: Yes to German OECD Principles
QA reports: yes
Drug, lot #, and % purity: CD 10503, lot PR4PAC02204A1, purity not specified

Methods
Strains/species/cell line: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100 and TA 102

Doses used in definitive study: 0 (vehicle), 10, 25, 50, 100, and 200 µg/plate

Basis of dose selection: Toxicity seen in the preliminary study at doses ≥200 µg/plate.

Negative controls: 80% PBS/20% DMSO (vehicle)

Positive controls: Sodium azide, 9-aminoacridine, 2-nitrofluorene, mitomycin C and 2-aminoanthracene

Incubation and sampling times: Standard Ames assay methodology

Results
Study validity: The positive and negative controls performed as expected so this study is considered acceptable for regulatory purposes.

Study outcome: No increase in mutations was found in the TA 1535 or TA 1537 plates. In strains TA 98, TA 100 and TA 102, a statistically and biologically, concentration-dependent increase in mutations was found. Increases of 2-3x the control values were found with and without S9 metabolic activation.
Sponsor's tables:

Without metabolic activation

Test substance (µg/plate)	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
PBS pH3 / DMSO	12	5	45	104	404
CD 10503					
10	11	5	54	122	457
25	13	5	65	140	557
50	14	8	98	130	617
100	15	10	114	246	1015
200	20 T	12 T	52 T	330	1152
Positive Controls					
NaN_3 5	1148	-	-	1222	-
9-AA 50	-	269	-	-	-
2-NF 10	-	-	666	-	-
MMC 0.5	-	-	-	-	1365

With metabolic activation

Test substance (µg/plate)	Mean Revertants/Plate *S. typhimurium*				
	TA 1535	TA 1537	TA 98	TA 100	TA 102
Negative Control					
PBS pH3 / DMSO	11	6	38	112	461
CD 10503					
10	13	11	55	119	503
25	14	8	63	141	537
50	15	11	79	163	577
100	16	10	84	242	857
200	22 T	8 T	46 T	330	1042
Positive Controls					
2-AA 4	124	65	389	791	-
2-AA 10	-	-	-	-	880

P: Precipitation T: Toxicity -: Not tested Underlined values are regarded as increased

Thus, CD 10503 is a bacterial mutagen under the conditions of this assay. The source of CD 10503 is suspected to be the excipient (b) (4) used in the extended release tablets (b) (4)

Study title: **CD 10503 (degradation product of pramipexole ER): Mechanistic follow-up mutagenicity study using the S. typhimurium/mammalian microsome assay (non-GLP); Study 07B016.1**

Key findings: All strains showed an increased revertant frequency (2-3x) with CD 10503. Formaldehyde elicited a revertant increase (2-4x) at all evaluable doses.

When CD 10503 and formaldehyde were co-incubated with formaldehyde dehydrogenase (FDH), disparate findings were observed. At high concentrations of FDH, the number of revertants was significantly reduced in the TA 100 plates at low and mid concentrations

of CD 10503. The revertant level was unaffected in the TA 102 plates. The reason for this discrepancy between strains is unknown.

When semicarbazide (SEM) was added as a "chemical trap" for the formaldehyde, the number of revertants was significantly reduced in TA 100 and TA 102. The sponsor concluded that "due to the identical mutagenic pattern and magnitude of CD 10503 and formaldehyde in bacteria and the effective inactivation mechanisms shown, the release of formaldehyde in aqueous solution of CD 10503 is regarded as the ultimate mutagenic agent." This provides indirect evidence at best.

However, given the level of CD 10503 in 4.5 mg pramipexole tablets, the exposure would be maximal at (b) (4) degradation level.

Strains/species/cell line: *Salmonella typhimurium* strains TA 98, TA 100, TA 102

Doses used in definitive study: 0 (vehicle), 10, 25, 50, 100 and 200 µg/plate of CD 10503; 0, 2, 5, 10, 25 or 50 µg/plate of formaldehyde

Basis of dose selection: GLP- compliant Ames assay on CD 10503 with positive findings in these 3 *Salmonella* strains. As mutagenicity was found with and without S9 activation, no S9 activation was used in this study.

Negative controls: Vehicle

Positive controls: Sodium azide, 2-nitrofluorene, mitomycin C

Incubation and sampling times: Standard Ames assay methodology

Results

Study validity: The positive and negative controls performed as expected so this study is considered acceptable for regulatory purposes.

Study outcome: All strains showed an increased revertant frequency (2-3x) with CD 10503.
Formaldehyde elicited a revertant increase (2-4x) at all evaluable doses.

When CD 10503 and formaldehyde were co-incubated with formaldehyde dehydrogenase [FDH] (Strains TA 100 and TA 102), disparate findings were observed. At high (0.5- 2.5 U/plate) concentrations of FDH, the number of revertants was significantly reduced in the TA 100 plates at low and mid doses of CD 10503. The revertant level was unaffected in the TA 102 plates for both CD 10503 and formaldehyde. The reason for this discrepancy between strains is unknown.

Table 8.1 *S. typhimurium* TA 100 (nonactivation) with CD 10503 and formaldehyde (FA): Inactivation by formaldehyde-dehydrogenase (FDH)

CD 10503			**FA**		**CD 10503 + FDH 0.5 U/plate**		**CD 10503 + FDH 2.5 U/plate**		**FA + FDH 0.5 U/plate**		**FA + FDH 2.5 U/pl.**
µg/ plate	**Rev. / plate**	**FA**	**µg/ plate**	**Rev. / plate**	**µg/ plate**	**Rev. / plate**	**Rev. / plate**	**FA**	**µg/ plate**	**Rev. / plate**	**Rev. / plate**
Control pH3	**131**		**Control pH3**	**131**	**Control pH3**	**143**	**134**		**Control pH3**	**143**	**143**
10	**138**	**≈ 1.6**			**10**	**135**	**139**	**≈ 1.6**			
25	**148**	**≈ 3.1**	**2**	**160**	**25**	**135**	**146**	**≈ 3.1**	**2**	**145**	**145**
50	**183**	**≈ 6.2**	**5**	**182**	**50**	**152**	**159**	**≈ 6.2**	**5**	**149**	**139**
100	**305**	**≈ 12.5**	**10**	**256**	**100**	**185**	**172**	**≈ 12.5**	**10**	**155**	**147**
200	**387**	**≈ 25**	**25**	**485**	**200**	**323**	**215**	**≈ 25**	**25**	**252**	**193**
			50	**258 T**					**50**	**382**	**242**

Historical Data: TA 100: 49 to 150 Underlined values are regarded as increased

Table 8.2 *S. typhimurium* TA 102 (nonactivation) with CD 10503 and formaldehyde (FA): Inactivation by formaldehyde-dehydrogenase (FDH)

CD 10503			FA		CD 10503 + FDH 1.0 U/plate		FA + FDH 1.0 U/plate	
µg/ plate	Rev. / plate	FA	µg/ plate	Rev. / plate	µg/ plate	Rev. / plate	µg/ plate	Rev. / plate
Control pH3	386		Control pH3	386	Control pH3	391	Control pH3	391
10	388	≈ 1.6			10	398		
25	418	≈ 3.1	2	425	25	430	2	394
50	517	≈ 6.2	5	502	50	582	5	469
100	611	≈ 12.5	10	653	100	865	10	572
200	742	≈ 25	25	1070	200	1417	25	1222
			50	1040			50	1630

Historical Data: Underlined values are regarded as increased

TA 102: 249 to 458

When semicarbazide (SEM) was added as a "chemical trap" for the formaldehyde, the number of revertants was significantly reduced in TA 100 and TA 102 (see sponsor's tables below).

Table 8.4 *S. typhimurium* TA 100 (nonactivation) with CD 10503 and formaldehyde (FA): Inactivation by semicarbazide (SEM)

CD 10503			FA		CD 10503 + SEM				FA + SEM			SEM	
µg/plate	Rev. / plate	FA	µg/plate	Rev. / plate	µg/plate		Rev. / plate	FA	µg/plate		Rev. / plate	µg/ plate	Rev. / plate
Control pH3	131		Control pH3	131	Control pH3		132		Control pH3		132	Control pH3	132
					CD	SEM			FA	SEM			
10	138	≈ 1.6			10	6.2	155	≈ 1.6				10	141
25	148	≈ 3.1	2	160	25	15.6	154	≈ 3.1	2	5	137	100	149
50	183	≈ 6.2	5	182	50	31.2	155	≈ 6.2	5	25	157	1000	216 T
100	305	≈ 12.5	10	256	100	62.5	163	≈ 12.5	10	50	147		
200	387	≈ 25	25	485	200	125	179	≈ 25	25	125	189		
			50	258 T					50	250	151 T		

Historical Data: TA 100: 49 to 150 Underlined values are regarded as increased

Table 8.5 *S. typhimurium* TA 102 (nonactivation) with CD 10503 and formaldehyde (FA): Inactivation by semicarbazide (SEM)

CD 10503			FA		CD 10503 + SEM				FA + SEM			SEM	
µg/plate	Rev. / plate	FA	µg/plate	Rev. / plate	µg/plate		Rev. / plate	FA	µg/plate		Rev. / plate	µg/ plate	Rev. / plate
Control pH3	408		Control pH3	408	Control pH3		407		Control pH3		407	Control pH3	408
					CD	SEM			FA	SEM			
10	431	≈ 1.6			10	6.2	443	≈ 1.6					
25	493	≈ 3.1	2	449	25	15.6	466	≈ 3.1	2	5	412	10	388
50	598	≈ 6.2	5	514	50	31.2	482	≈ 6.2	5	25	418		
100	778	≈ 12.5	10	589	100	62.5	534	≈ 12.5	10	50	449	50	442
200	922	≈ 25	25	790	200	125	635	≈ 25	25	125	477	100	440
			50	1043					50	250	449	250	381
												500	315 T

Historical Data: TA 102: 249 to 458 Underlined values are regarded as increased

The sponsor concluded that "due to the identical mutagenic pattern and magnitude of CD 10503 and formaldehyde in bacteria and the effective inactivation mechanisms shown, the release of formaldehyde in aqueous solution of CD 10503 is regarded as the ultimate mutagenic agent." This provides indirect evidence at best.

Table 8.3 *S. typhimurium* TA 98 (nonactivation) with CD 10503 and formaldehyde (FA): Inactivation by semicarbazide (SEM)

CD 10503			FA		CD 10503 + SEM				FA + SEM			SEM	
µg/plate	Rev. / plate	FA	µg/plate	Rev. / plate	µg/plate		Rev. / plate	FA	µg/plate		Rev. / plate	µg/ plate	Rev. / plate
Control pH3	26		Control pH3	26	Control pH3		27		Control pH3		27	Control pH3	26
					CD	SEM			FA	SEM			
10	29	≈ 1.6			10	6.2	35	≈ 1.6					
25	41	≈ 3.1	2	47	25	15.6	31	≈ 3.1	2	5	35	10	45
50	69	≈ 6.2	5	63	50	31.2	41	≈ 6.2	5	25	34		
100	94	≈ 12.5	10	89	100	62.5	84	≈ 12.5	10	50	51	50	49
200	65 T	≈ 25	25	96	200	125	60 T	≈ 25	25	125	66	100	33 T
			50	12 T					50	250	56	250	14 T
												500	12 T

Historical Data: TA 98: 16 to 68 Underlined values are regarded as increased

Study title: **Product Z (degradation product of pramipexole): Mutagenicity study for chromosomal aberrations in human lymphocytes in vitro**

Key findings: Incubation of human lymphocytes with the pramipexole degradation products Z and V did not induce increased chromosomal aberrations when tested at 30-300 µg/mL without S9 and 30-1000 µg/mL with S9 for 4 hrs. However, when these products were tested at 100 µg/mL without S9 for 24 hrs, a statistically significant

increase in aberration frequencies (4.0% compared to 0.5% in controls) was determined, but the mixture elicited a decrease in the mitotic index (~62% of control value) at 100 µg/mL. Catechol was present in the products at 1.4% and it alone elicited an increased aberration frequency (without S9, 24 hr harvest; ≤8% increase). No chromosomal rearrangements were found in any of the evaluated cultures. Therefore, under the conditions of this assay, a mixture containing pramipexole degradation products Z+V did not induce reproducible increases in chromosomal aberrations with or without S9 activation at doses up to those eliciting cytotoxicity.

Study no.: 07B127 or U08-1564-01

Volume and page #: Electronic submission
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co, KC, Biberach an der Riss, Germany
Date of study initiation: 9/24/07
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: The test article was a combination of catechol (1.4% of the mixture), Product Z and Product V, lot #PR2GFK02138PA2

Methods
Strains/species/cell line: Lymphocytes from "healthy volunteers" (further details not provided

Doses used in definitive study: 0, 30, 100 or 300 µg/mL without S9 and 0, 30, 100, 300 or 1000 µg/mL with S9 for Product Z+V. Catechol was added at 5 µg/mL for cultures without S9.
Confirmatory study: Product Z+V at 10, 30 or 100 µg/mL with harvest after 72 hrs and 100 and 300 µg/mL with harvest at 96 hrs. Catechol was tested at 1 and 2.5 µg/mL with a harvest at 72 hrs.

Basis of dose selection: Cytotoxicity at concentration-dependent levels

Negative controls: Vehicle

Positive controls: Adriamycin without S9; cyclophosphamide with S9

Incubation and sampling times: Standardized methodology

Results
Study validity: The test articles were soluble at all concentrations. The positive (22.0-40.5% for adriamycin; 33.5% for cyclophosphamide depending on the length of incubation and S9) and negative (1%) controls performed as expected so this study is considered adequate for regulatory purposes.

Study outcome: Cultures treated with Product Z+V turned black at concentrations of ≥300 µg/mL.

Without S9 activation: Product Z+V elicited a decrease in the mitotic index (~65% of control value) at 300 µg/mL and -72% at 1000 µg/mL without S9 in the 4 hr exposure scenario. Similarly, the mixture elicited a decrease in the mitotic index (~62% of control value) at 100 µg/mL and 18% at 399 and 18% at 300 µg/mL without S9 in the 24 hr exposure scenario. Inhibitions of 33% (at 100 µg/mL) and 75% (at 300 µg/mL) were noted in cultures without S9 harvested at 96 hrs. No concentration-dependent increases in mean aberrations were discerned when cells were incubated without S9 for 4 hrs at doses ≤300 µg/mL. However, with a 24-hr incubation (Product Z+V without S9), a statistically significant increase (4%) in aberration frequency was noted when compared to controls and the laboratory's historical controls. No chromosomal rearrangements were observed but they did report chromatid breaks, acentric fragments and chromosomal breakage.

At the 96 hr harvest, increased aberrations (6.4%) were seen at the 300 µg/mL concentration. The low mitotic index (25% of control value) makes the interpretation difficult (due to the cytotoxicity) and no chromosomal rearrangements were detected.

Catechol did not induce aberrations when tested at 5 µg/mL without S9 for 4 hrs. When it was tested for 24 hrs, it induced increases of 6% (at 1 µg/mL) and 8% (at 2.5 µg/mL) without S9. The sponsor concluded that the 1.4 µg/mL catechol in the 100 µg/mL Product Z+V "contributed to the weak increase in the aberration frequency observed with the mixture". No chromosomal rearrangements were observed but they did report chromatid breaks, acentric fragments and chromosomal breakage.

When the Product Z+V mixture was tested in the presence of S9 metabolic activation, declines in the mitotic indices were ~97% (at 300 µg/mL) and 32% (at 1000 µg/mL) at the 4 hr harvest.

With S9 activation: As in the cultures without S9, the mitotic index was decreased (32% of control value) only in the 1000 µg/mL cultures at 4 hrs but increased mean aberration frequency (5.9%) was described. The increase may have been due to the excessive toxicity. No chromosomal rearrangements were found.

When Product Z+V was added at ≤1000 µg/mL without metabolic activation, neither effects on mitotic index nor chromosomal aberrations was found.

Table 8.1: 2 Summary Structural Chromosomal Aberrations (% Aberrant Cells excl. Gaps)

Substance (µg/mL)	Culture No.	MI %	Treatment 4 h Harvest: 72th h	MI %	Treatment 24 h Harvest: 72th h
Controls: **Negative** Medium 1A	A		1.0		1.0
	B		1.0		2.0
	Mean	**100**	**1.0**	**100**	**1.5**
ADR 0.075 0.05	A		18.0		19.0
	B		22.0		17.0
	Mean	**84**	**20.0***	**87**	**18.0***
Catechol **1**	A				6.0
	B				6.0
	Mean	**na**	**na**	**125**	**6.0**
2.5	A				8.0
	B				8.0
	Mean	**na**	**na**	**113**	**8.0**
5	A		2.0		
	B		3.0		
	Mean	**97**	**2.5**	**na**	**na**

na: not analyzed
*: Significantly different from the vehicle control ($p \leq 0.05$)
Historical negative control values (% aberrant cells excl. gaps): 0.9 (range 0-2.5)

Table 8.1: 7 **Chromosomal Aberrations in Human Lymphocytes without Metabolic Activation**

Substance: **Pramipexol/Product Z** Donor: (b) (6)
Treatment: **48h –72h = 24 h** Culture: 15–18 October 2007
Harvesting: **72h**

Substance (µg/mL)	MI %	Culture No.	Cells scored	Type and Incidence of Aberrations							Aberrant Cells Total		Aberrant Cells %	
				g	ctb	cte	ace	td	csb	cse	Incl. Gaps	Excl. Gaps	Incl. Gaps	Excl. Gaps
Controls:														
		A	100	3			1				4	1	4.0	1.0
Negative		B	100	1							1	0	1.0	0
Medium 1A	**100**	**A+B**	**200**	**4**	**0**	**0**	**1**	**0**	**0**	**0**	**5**	**1**	**2.5**	**0.5**
		A	100	4	8	10	8	1	3		34	30	34.0	30.0
		B	100	3	5	14	5	1	3		31	28	31.0	28.0
Positive														
ADR 0.05	**31**	**A+B**	**200**	**7**	**13**	**24**	**13**	**2**	**6**	**0**	**65**	**58**	**32.5**	**29.0**
Product Z														
		A	100	1	2						3	2	3.0	2.0
		B	100	1			2				3	2	3.0	2.0
10	**86**	**A+B**	**200**	**2**	**2**	**0**	**2**	**0**	**0**	**0**	**6**	**4**	**3.0**	**2.0**
		A	100	3	2						5	2	5.0	2.0
		B	100		2		1				3	3	3.0	3.0
30	**72**	**A+B**	**200**	**3**	**4**	**0**	**1**	**0**	**0**	**0**	**8**	**5**	**4.0**	**2.5**
		A	100	3	1				1		5	2	5.0	2.0
		B	100	1	5		1				7	6	7.0	6.0
100 T	**62**	**A+B**	**200**	**4**	**6**	**0**	**1**	**0**	**1**	**0**	**12**	**8**	**6.0**	**4.0**
		A	15								Not evaluable			
		B	41								Not evaluable			
300 TC	**18**	**A+B**	**56**											

T: Toxicity (mitotic inhibition and/or cellular toxicity)
C: Black coloured culture during treatment

Table 8.2: 3 Chromosomal Aberrations in Human Lymphocytes with Metabolic Activation

Substance: **Product Z** Donor: (b) (6)
Treatment: **48th–52th h = 4 h** Culture: 24–27 September 2007
Harvesting: **72th h**

Substance (µg/mL)	MI %	Culture No.	Cells scored	Type and Incidence of Aberrations							Aberrant Cells Total		Aberrant Cells %	
				g	ctb	cte	ace	td	csb	cse	Incl. Gaps	Excl. Gaps	Incl. Gaps	Excl. Gaps
Controls:														
		A	100	1			1				2	1	2.0	1.0
Negative		B	100								0	0	0	0
Medium 1A	100	A+B	200	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**2**	**1**	**1.0**	**0.5**
		A	100	5	13	10	8		4		40	35	40.0	35.0
Positive		B	100	5	2	17	5	1	7		37	32	37.0	32.0
CP 7	73	A+B	200	**10**	**15**	**27**	**13**	**1**	**11**	**0**	**77**	**67**	**38.5**	**33.5**
Product Z														
		A	100	1	2						3	2	3.0	2.0
		B	100		1						1	1	1.0	1.0
30	91	A+B	200	**1**	**3**	**0**	**0**	**0**	**0**	**0**	**4**	**3**	**2.0**	**1.5**
		A	100								0	0	0	0
		B	100	1	1				1		3	2	3.0	2.0
100	95	A+B	200	**1**	**1**	**0**	**0**	**0**	**1**	**0**	**3**	**2**	**1.5**	**1.0**
		A	100	3	1						4	1	4.0	1.0
		B	100	2							2	0	2.0	0
300 C	97	A+B	200	**5**	**1**	**0**	**0**	**0**	**0**	**0**	**6**	**1**	**3.0**	**0.5**
		A	67	1			1		3		5	4	7.5	6.0
		B	34				1		1		2	2	5.9	5.9
1000 TC	32	A+B	101	**1**	**0**	**0**	**2**	**0**	**4**	**0**	**7**	**6**	**6.9**	**5.9**

T: toxicity (mitotic inhibition and/or cellular toxicity)

C: Black coloured culture during treatment

Study title: **Product Z (degradation product of pramipexole): Mutagenicity study using the mouse lymphoma (L5178Y) assay**

Key findings: Under the conditions of this study, Product Z+V and catechol, individually and together, increased the incidence of mutant frequencies in mouse lymphoma L5178Y cells for both large and small colonies. Product Z+V elicited significant decreases in relative growth compared to control cultures.

Study no.: 07B134 or U08-1365-01
Volume and page #: Electronic submission
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany
Date of study initiation: 10/9/07
GLP compliance: Yes
QA reports: yes

Drug, lot #, and % purity: Mixture of Product Z (7.9%) and V (7.6%), lot # PR2GFK02138PA2 and catechol lot #04013HE

Methods
Strains/species/cell line: Mouse lymphoma L5178Y $tk^{+/-}$ cells

Doses used in definitive study: Without S9 (4 hr incubation): 10, 100, 150, 200, 225 and 250 µg/mL; Without S9 (24 hr incubation): 1, 10, 100, 150, and 200 µg/mL
With S9 (4 hr incubation): 10, 50, 100, 250, 300 and 500 µg/mL

Basis of dose selection: Dose range-finding assay at doses of 100-5000 µg/mL with decreased growth (<10% compared to controls) noted at all doses ≥250 µg/mL.

Negative controls: RPMI 1640 culture medium

Positive controls: 0.1 µg/mL 4-nitroquinoline-N-oxide without S9; benzo[a]pyrene with S9

Incubation and sampling times: Standardized methodology

Results
Study validity: The 4-NQO cultures had 66-80% relative growth and the B(a)P decreased the relative growth to 11-27% after 4 hrs of treatment. The positive and the negative controls performed as anticipated so this study is considered adequate for regulatory purposes.

Study outcome: Products Z+V continued to degrade in solution and the catechol levels increased 1.5x when incubated in culture medium for 4 hrs.

Table 3.2.1 Product Z (degradation product of pramipexole): Mutagenicity study using the mouse lymphoma (L5178Y) assay

Substance	0h	1h	2h	3h	4h
Product Z	7.5 % (w/w)	7.2 % (w/w)	6.9 % (w/w)	6.6 % (w/w)	6.1 % (w/w)
Product V	6.7 % (w/w)	6.5 % (w/w)	6.2 % (w/w)	5.9 % (w/w)	5.5 % (w/w)
Catechol	1.7 % (w/w)	1.8 % (w/w)	1.9 % (w/w)	2.2 % (w/w)	2.6 % (w/w)

A steep concentration-dependent cytotoxicity was appreciated after 4 hrs of incubation whether with or without S9 added. The relative total growth (RTG) was 17% at 200 (without S9) -250 (with S9) µg/mL and remained about the same levels at up to the highest concentrations tested. RTGs were 87% of control values at 10 µg/mL and 10% at 100 µg/mL when no metabolic activation was present. Catechol provided RTGs of 42% and 53% at 5 µg/mL without and with S9, respectively.

Without S9: 4 hr incubation: Product Z+V induced a concentration-dependent increase in mutant frequency (2.5x at 100 µg/mL; 3.3x at 200 µg/mL) with increases in both large

and small colonies. Catechol also induced an increase at 2.5 µg/mL (1.7x compared to controls) and 5 µg/mL (2.3x). Both large and small colonies were increased.

24 hr incubation: An 11% increase in mutant frequency was found at 100 µg/mL but the relative growth was 7% (1 culture) and the frequency increased for both large and small colonies.

With S9: There was a concentration-dependent increase in mutant frequency when incubation was 4 hrs with Product Z+V. The increase was 3x at 100 µg/mL and 6.2x at 250 µg/mL but the relative growth was 17%. Both large and small colonies were increased.
Catechol elicited an increased mutant frequency at 1.5x at 2.5 µg/mL and 2.4x at 5 µg/mL. Both large and small colonies were increased. The sponsor surmised that the oxidative mechanism coupled with the S9 and the "complexity of the mixture" "might be associated with a higher mutant frequency for catechol in the mixture compared to catechol tested alone".

Nonactivation (4 h exposure) - Summary of results

Test Substance	Culture	Cytotoxicity		Mutant frequencies per 10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: Medium	1	100	100	46	74	124
	2	100	100	51	83	141
	Mean	**100**	**100**	**49**	**79**	**133**
Mixture (µg/mL)						
10	1	106	94	44	93	142
	2	89	88	40	55	100
	Mean	**98**	**91**	**42**	**74**	**121**
100	1	52	55	139	146	325
	2	43	46	129	172	336
	Mean	**48**	**51**	**134**	**159**	**331**
150	1	27	26	150	178	373
	2	21	25	154	160	346
	Mean	**24**	**26**	**152**	**169**	**360**
200	1	18	17	213	215	467
	2	16	16	189	170	414
	Mean	**17**	**17**	**201**	**193**	**441**
225	1	17	14	193	214	479
	2	15	11	225	280	593
	Mean	**16**	**13**	**209**	**247**	**536**
250	1	21	13	186	201	440
	2	15	15	146	190	407
	Mean	**18**	**14**	**166**	**196**	**424**
Positive Control: 4-NQO 0.1	1	76	78	262	306	726

RS Relative survival
RTG Relative total growth

Nonactivation (4 h exposure) - Summary of results

Test Substance	Culture	Cytotoxicity		Mutant frequencies per 10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: Medium	1	100	100	44	139	199
	2	100	100	51	124	190
	Mean	**100**	**100**	**48**	**132**	**195**
Catechol (µg/mL)						
0.1	1	108	87	52	140	198
	2	93	105	50	129	188
	Mean	**101**	**96**	**51**	**135**	**193**
0.5	1	111	101	72	130	213
	2	86	78	66	176	258
	Mean	**99**	**90**	**69**	**153**	**236**
1	1	100	84	69	140	224
	2	85	88	59	161	235
	Mean	**93**	**86**	**64**	**151**	**230**
2.5	1	80	80	109	180	342
	2	85	82	104	175	312
	Mean	**83**	**81**	**107**	**178**	**327**
5	1	42	45	131	265	440
	2	40	39	161	232	454
	Mean	**41**	**42**	**146**	**249**	**447**
Positive Control: 4-NQO 0.1	1	73	80	280	324	793

RS Relative survival
RTG Relative total growth

Nonactivation (24 h exposure) - Summary of results

Test Substance	Culture	Cytotoxicity		Mutant frequencies per 10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: Medium	1	100	100	25	69	98
	2	100	100	26	52	81
	Mean	**100**	**100**	**26**	**61**	**90**
Mixture (µg/mL)						
1	1	127	106	22	68	94
	2	120	112	25	64	93
	Mean	**124**	**109**	**24**	**66**	**94**
10	1	97	83	60	81	149
	2	125	90	29	78	107
	Mean	**111**	**87**	**45**	**80**	**128**
100	1	25	13	390	422	1022
	2	2	0	ND	ND	ND
	Mean	**14**	**7**	**390**	**422**	**1022**
150	1	0	0	ND	ND	ND
	2	3	1	ND	ND	ND
	Mean	**2**	**1**			
200	1	1	0	ND	ND	ND
	2	0	ND	ND	ND	ND
	Mean	**1**	**0**			
Positive Control: 4-NQO 0.1	1	70	66	234	433	876

RS Relative survival
RTG Relative total growth
ND Not done

With metabolic activation (4 h exposure) - Summary of results

Test Substance	Culture	Cytotoxicity		Mutant frequencies per 10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: Medium	1	100	100	30	101	139
	2	100	100	29	93	126
	Mean	**100**	**100**	**30**	**97**	**133**
Mixture (µg/mL)						
10	1	104	91	31	127	166
	2	116	116	26	93	125
	Mean	**110**	**104**	**29**	**110**	**146**
50	1	83	65	63	193	265
	2	94	95	44	121	172
	Mean	**89**	**80**	**54**	**157**	**219**
100	1	75	57	98	284	437
	2	82	76	108	224	362
	Mean	**79**	**67**	**103**	**254**	**400**
250	1	33	13	245	437	829
	2	39	21	271	393	812
	Mean	**36**	**17**	**258**	**415**	**821**
300	1	24	12	263	429	853
	2	34	17	257	359	761
	Mean	**29**	**15**	**260**	**394**	**807**
500	1	18	7	303	718	1269
	2	23	12	294	471	1069
	Mean	**21**	**10**	**299**	**595**	**1169**
Positive Control: B(*a*)P 2	1	29	11	631	1134	2474

RS Relative survival
RTG Relative total growth

With metabolic activation (4 h exposure) - Summary of results

Test Substance	Culture	Cytotoxicity		Mutant frequencies per 10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: Medium	1	100	100	36	146	195
	2	100	100	40	138	192
	Mean	**100**	**100**	**38**	**142**	**194**
Catechol (µg/mL)						
0.1	1	90	104	33	122	160
	2	116	90	44	112	164
	Mean	**103**	**97**	**39**	**117**	**162**
0.5	1	105	102	50	159	229
	2	103	87	49	114	170
	Mean	**104**	**95**	**50**	**137**	**200**
1	1	88	76	63	185	260
	2	101	75	40	164	214
	Mean	**95**	**76**	**52**	**175**	**237**
2.5	1	96	81	100	170	296
	2	92	75	82	161	272
	Mean	**94**	**78**	**91**	**166**	**284**
5	1	43	54	132	246	433
	2	72	51	135	274	491
	Mean	**58**	**53**	**134**	**260**	**462**
Positive Control: B(*a*)P	1	34	27	437	664	1748

RS Relative survival
RTG Relative total growth

Study title: **CD 10503 (degradation product of pramipexole): Mutagenicity study from chromosomal aberrations in human lymphocytes in vitro**

Key findings: Statistically and biologically significant increases in chromosomal aberrations were found in human lymphocytes treated with CD 10503, a degradant of pramipexole. The sponsor considered the formaldehyde to be the source of the positive response but their conclusion is not supported by the data. They state that the exposure to CD 10503 when administering 4.5 mg Mirapex® ER™ would be (b) (4) and that this level is significantly below the acceptable EPA level of 10 mg/day. This conclusion appears to be reasonable.

Study no.: 07B160 or U08-1775-01

Volume and page #: Electronic submission
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany
Date of study initiation: 3/3/08
GLP compliance: Yes
QA reports: yes

Drug, lot #, and % purity: CD 10503 (degradant of pramipexole ER found at (b) (4) lot # PR4PAC02204A1 at 54.1% potency. The sponsor suggests that CD 10503 is formed from (b) (4) (excipient) reacting with pramipexole's (b) (4) The structure of CD 10503 is below:

(b) (4)

Methods

Strains/species/cell line: Human lymphocytes

Doses used in definitive study: 0 (PBS/DMSO at 80:20), 5, 25, 50 and 60 µg/mL without S9 in the cultures and 0, 5, 50 and 60 µg/mL with S9 added to the cultures.

Basis of dose selection: Range-finding study where CD 10503 elicited a significant decrease in the mitotic index at ≥50 µg/mL.

Negative controls: PBS/DMSO (80%/20%)

Positive controls: Formaldehyde at 10 (without S9) and 15 (with S9) µg/mL as a reference substance; adriamycin and cyclophosphamide

Incubation and sampling times: Standardized methodology with 4 hr incubation

Results

Study validity: The positive and negative controls performed as anticipated. This study is considered adequate for regulatory purposes. Adriamycin elicited increased aberrations at 27%. Cyclophosphamide elicited increased aberrations at 43%.

Study outcome: **Without S9**: There was a dose-dependent decrease in the mitotic index at 50 µg/mL of CD 10503 (61% compared to controls) and 60 µg/mL (26% compared to controls) in the cultures. A statistically and biologically significant increase in the mean aberration frequency was found at 50 µg/mL (40.5%) and 60 µg/mL (61.7%) compared to the negative controls (3.0%; historical control range: 0- 2.5%).
Formaldehyde under similar conditions decreased the mitotic index at 10 µg/mL (72% of controls) and 15 µg/mL (28% of controls). There was a statistically and biologically significant increase in the mean aberration frequency was found at 10 µg/mL (24.0%) and 15µg/mL (62.5%) compared to the negative controls (3.0%; historical control range: 0- 2.5%).

Substance (µg/mL)	Culture No.	MI %	Treatment 4 h Harvest: 72th h
Controls:			
	A		4.0
Negative	B		2.0
PBS pH 3 / DMSO	**Mean**	**100**	**3.0**
Positive	A		27.0
ADR	B		27.0
0.075	**Mean**	**76**	**27.0***
			p<0.0001
CD 10503	A		1.0
	B		2.0
5	**Mean**	**98**	**1.5**
			p=0.5028
	A		4.0
	B		8.0
25	**Mean**	**81**	**6.0**
			p=0.2271
	A		33.0
	B		48.0
50 T	**Mean**	**61**	**40.5***
			p<0.0001
	A		58.0
	B		70.7
60 T	**Mean**	**26**	**61.7***
			p<0.0001

T: Toxicity
*: Significantly different from the vehicle control ($p \leq 0.05$)
Historical negative control values (% aberrant cells excl. gaps): Mean 0.9 %, Range 0-2.5 %

Table 2 Summary Structural Chromosomal Aberrations (% Aberrant Cells excl. Gaps) with Formaldehyde in the Absence of Metabolic Activation

Substance (µg/mL)	Culture No.	MI %	Treatment 4 h Harvest: 72th h
Controls:			
	A		4.0
Negative	B		2.0
PBS pH 3 / DMSO	**Mean**	**100**	**3.0**
Positive	A		27.0
ADR	B		27.0
0.075	**Mean**	**76**	**27.0***
			p<0.0001
Formaldehyde	A		22.0
	B		26.0
10 T	**Mean**	**62**	**24.0***
			p<0.0001
	A		63.0
	B		61.4
15 T	**Mean**	**28**	**62.5***
			p<0.0001

T: Toxicity
*: Significantly different from the vehicle control ($p \leq 0.05$)
Historical negative control values (% aberrant cells excl. gaps): Mean 0.9 %, Range 0-2.5 %

Table 5 Type and Incidence of Chromosomal Aberrations in Human Lymphocytes with CD 10503 in the Absence of Metabolic Activation

Substance: **CD 10503** Donor: (b) (6)
Treatment: **48^{th} –52^{th} h = 4 h** Culture: 03-06 March 2008
Harvesting: **72^{th} h**

Substance (µg/mL)	MI %	Culture No.	Cells scored	Type and Incidence of Aberrations							Aberrant Cells Total		Aberrant Cells %	
				g	ctb	csb	ace	td	cte	cse	Incl. Gaps	Excl. Gaps	Incl. Gaps	Excl. Gaps
Controls:														
		A	100	2	1		3				6	4	6.0	4.0
Negative		B	100	3	1		1				5	2	5.0	2.0
PBS pH 3 / DMSO	100	A+B	200	**5**	2	**0**	4	**0**	**0**	**0**	**11**	**6**	**5.5**	**3.0**
		A	100	5	8	1	15	1	2		32	27	32.0	27.0
Positive		B	100	5	10	1	12		4		32	27	32.0	27.0
ADR 0.075	76	A+B	200	**10**	**18**	2	**27**	**1**	**6**	**0**	**64**	**54**	**32.0**	**27.0**
CD 10503														
		A	100	1			1				2	1	2.0	1.0
		B	100	2	1		1				4	2	4.0	2.0
5	98	A+B	200	**3**	1	**0**	2	**0**	**0**	**0**	**6**	**3**	**3.0**	**1.5**
		A	100	2	2		2				6	4	6.0	4.0
		B	100	1		3	5				9	8	9.0	8.0
25	81	A+B	200	**3**	2	**3**	**7**	**0**	**0**	**0**	**15**	**12**	**7.5**	**6.0**
		A	100	4	10	2	4		17		37	33	37.0	33.0
		B	100	3	12	6	5		25		51	48	51.0	48.0
50 T	61	A+B	200	**7**	22	**8**	**9**	**0**	42	**0**	**88**	**81**	**44.0**	**40.5**
		A	100	5	13	6	7		32		63	58	63.0	58.0
		B	41	2	10	4	5		10		31	29	75.6	70.7
60 T	26	A+B	141	**7**	23	**10**	**12**	**0**	42	**0**	**94**	**87**	**66.7**	**61.7**

T: Toxicity (mitotic inhibition and/or cellular toxicity)

With S9: There was a dose-dependent decrease in the mitotic index at 50 µg/mL (80% compared to controls) and 60 µg/mL (40% compared to controls) in the cultures. With S9 added, there was a statistically and biologically significant increase in the mean aberration frequency was found at 50 µg/mL (20.5%) and 60 µg/mL (44.5%) compared to the negative controls (3.0%; historical control range: 0- 2.5%).Formaldehyde under similar conditions decreased the mitotic index at 10 µg/mL (72% of controls) and 15 µg/mL (22% of controls). Formaldehyde elicited statistically and biologically significant increase in the mean aberration frequency was found at 10 µg/mL (10.0%) and 60 µg/mL (40.0%) compared to the negative controls (3.0%; historical control range: 0- 2.5%).

Table 7 Summary Structural Chromosomal Aberrations (% Aberrant Cells excl. Gaps) with CD 10503 in the Presence of Metabolic Activation

Substance (µg/mL)	Culture No.	MI %	Treatment 4 h Harvest: 72th h
Controls:			
	A		2.0
Negative	B		4.0
PBS pH 3 / DMSO	**Mean**	**100**	**3.0**
	A		46.0
Positive	B		40.0
CP 7	**Mean**	**80**	**43.0***
			p<0.0001
CD 10503			
	A		1.0
	B		0
5	**Mean**	**94**	**0.5**
			p=0.1217
	A		22.0
	B		19.0
50	**Mean**	**80**	**20.5***
			p<0.0001
	A		42.0
	B		47.0
60 T	**Mean**	**40**	**44.5***
			p<0.0001

T: Toxicity (mitotic inhibition and/or cellular toxicity)
*: Significantly different from the vehicle control ($p \leq 0.05$)
Historical negative control values (% aberrant cells excl. gaps): Mean 0.7 %, Range 0-3.0 %

Table 8 Summary Structural Chromosomal Aberrations (% Aberrant Cells excl. Gaps) with Formaldehyde in the Presence of Metabolic Activation

Substance (µg/mL)	Culture No.	MI %	Treatment 4 h Harvest: 72th h
Controls:			
	A		2.0
Negative	B		4.0
PBS pH 3 / DMSO	**Mean**	**100**	**3.0**
	A		46.0
Positive	B		40.0
CP 7	**Mean**	**80**	**43.0***
			p<0.0001
Formaldehyde			
	A		7.0
	B		13.0
10 T	**Mean**	**72**	**10.0***
			p=0.0073
	A		36.0
	B		44.0
15 T	**Mean**	**22**	**40.0***
			p<0.0001

T: Toxicity (mitotic inhibition and/or cellular toxicity)
*: Significantly different from the vehicle control ($p \leq 0.05$)
Historical negative control values (% aberrant cells excl. gaps): Mean 0.7 %, Range 0-3.0 %

Table 11 Type and Incidence of Chromosomal Aberrations in Human Lymphocytes with CD 10503 in the Presence of Metabolic Activation

Substance: CD 10503 Donor: (b) (6)
Treatment: 48^{th} –52^{th} h = 4 h Culture: 05-06 March 2008
Harvesting: 72^{th} h

Substance (µg/mL)	MI %	Culture No.	Cells scored	Type and Incidence of Aberrations							Aberrant Cells Total		Aberrant Cells %	
				g	ctb	csb	ace	td	cte	cse	Incl. Gaps	Excl. Gaps	Incl. Gaps	Excl. Gaps
Controls:														
		A	100	5	1		1				7	2	7.0	2.0
Negative		B	100	2	1	1	2				6	4	6.0	4.0
PBS pH 3 / DMSO	100	A+B	200	**7**	2	**1**	**3**	0	0	0	**13**	**6**	**6.5**	**3.0**
		A	100	6	17	6	6		17		52	46	52.0	46.0
Positive		B	100	9	10	2	5		23		49	40	49.0	40.0
CP 7	80	A+B	200	**15**	**27**	**8**	**11**	0	**40**	0	**101**	**86**	**50.5**	**43.0**
CD 10503														
		A	100	3	1						4	1	4.0	1.0
		B	100	4							4	0	4.0	0
5	94	A+B	200	**7**	1	0	0	0	0	0	**8**	**1**	**4.0**	**0.5**
		A	100	3	4	3	2		13		25	22	25.0	22.0
		B	100	6	5	3	3		8		25	19	25.0	19.0
50	80	A+B	200	**9**	**9**	**6**	**5**	0	21	0	**50**	**41**	**25.0**	**20.5**
		A	100	5	12	5	4		21		47	42	47.0	42.0
		B	100	7	8	8	2		29		54	47	54.0	47.0
60 T	40	A+B	200	**12**	**20**	**13**	**6**	0	**50**	0	**101**	**89**	**50.5**	**44.5**

T: toxicity (mitotic inhibition and/or cellular toxicity)

Table 12 Type and Incidence of Chromosomal Aberrations in Human Lymphocytes with Formaldehyde in the Presence of Metabolic Activation

Substance: Formaldehyde Donor: (b) (6)
Treatment: 48th –52th h = 4 h Culture: 03-06 March 2008
Harvesting: 72th h

Substance (µg/mL)	MI %	Culture No.	Cells scored	Type and Incidence of Aberrations							Aberrant Cells Total		Aberrant Cells %	
				g	ctb	csb	ace	td	cte	cse	Incl. Gaps	Excl. Gaps	Incl. Gaps	Excl. Gaps
Controls:														
		A	100	5	1		1				7	2	7.0	2.0
Negative		B	100	2	1	1	2				6	4	6.0	4.0
PBS pH 3 / DMSO	100	A+B	200	**7**	**2**	**1**	**3**	**0**	**0**	**0**	**13**	**6**	**6.5**	**3.0**
		A	100	6	17	6	6		17		52	46	52.0	46.0
Positive		B	100	9	10	2	5		23		49	40	49.0	40.0
CP 7	80	A+B	200	**15**	**27**	**8**	**11**	**0**	**40**	**0**	**101**	**86**	**50.5**	**43.0**
Formaldehyde														
		A	100		3		3		1		7	7	7.0	7.0
		B	100		5	1	7				13	13	13.0	13.0
10 T	72	A+B	200	**0**	**8**	**1**	**10**	**0**	**1**	**0**	**20**	**20**	**10.0**	**10.0**
		A	100	5	10	6	2		18		41	36	41.0	36.0
		B	100	4	10	7	2		25		48	44	48.0	44.0
15 T	22	A+B	200	**9**	**20**	**13**	**4**	**0**	**43**	**0**	**89**	**80**	**44.5**	**40.0**

T: toxicity (mitotic inhibition and/or cellular toxicity)

The sponsor suggested that the lower aberration frequencies with CD 10503, compared to the results with formaldehyde, may have been due to pramipexole acting as a reaction substrate for the formaldehyde and/or the cytotoxicity found with formaldehyde at 15 µg/mL. They identified formaldehyde as the "primary source for the positive clastogenic response of CD 10503 in this chromosomal aberration study in human lymphocytes". This evaluation is not consistent with the data.

Study title: **Pramipexole Product Z (degradation product): Mutagenicity study using the mouse lymphoma (L5178Y) assay**

Key findings: A statistically and biologically significant increase in mutant frequencies, large and small colonies, was found at doses ≥100 µg/mL. The increases were 2.4, 3.2 and 2.9x at 100, 200 and 300 µg/mL without S9 and 3.0, 3.4 and 3.5x with S9, respectively, when compared to controls. The sponsor suggested that Product Z is negative in the Ames assay and the rat micronucleus assay so they attribute the positive findings to catechol and not the pramipexole. Their conclusion was: "All the mutant frequencies seen with 100 µg/mL pramipexole Product Z releasing continuously catechol are comparable to those with 5 µg/mL catechol alone taking the major difference in continuous release versus catechol being present at fixed concentrations and the biological variance of this test into account." The lack of importance for this finding is not a reasonable conclusion as the catechol will be present in the administered product

upon its degradation. They performed a "compound-specific risk assessment" and determined that the maximum daily exposure to catechol after being given pramipexole as an extended release product would be significantly less than the exposure to catechol from food (70-2500x higher).

Study no.: 08B082 or U08-1841-01
Volume and page #: Electronic submission
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany
Date of study initiation: 5/6/08
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: Pure Product Z, batch PR2GFK02340PA4 at 82.6% purity as the HCl
After culturing the Product Z for 4 hrs, the sponsor calculated that 1.8, 3.8 and 5.7 µg/mL catechol would have formed after incubation for 1, 2 or 3 hrs, respectively. The findings for the non-GLP stability assay for Product Z are as follows:

Substance	Concentration in the treatment medium [%(w/w)]					
	0 h	0.5 h	1 h	2 h	3 h	4 h
Product Z	54.3	52.4	49.9	45.7	41.2	35.1
Product V	0.03	0.07	0.09	0.14	0.20	0.26
Catechol	0.0	0.74	1.9	3.7	5.6	8.0
Thiourea	0.06	0.82	1.6	2.8	4.3	6.0

Methods
Strains/species/cell line: Mouse lymphoma L5178Y cells

Doses used in definitive study: With or without S9: 0, 10, 30, 100, 200 or 300 µg/mL

Basis of dose selection: This study was conducted to determine if pure Product Z would induce the same findings as the previously conducted mouse lymphoma assay (positive mouse lymphoma assay with Product Z+V producing catechol). A dose range-finding study was performed with doses of 10-300 µg/mL Product Z as free base. Significant decreases in relative suspension growth were found at doses ≥100 µg/mL.

An additional study (U08-1840-01) was performed with Product V (epimer of Product Z) and the epimer also elicited significant increases in mutant frequency of both large and small colonies.

Negative controls: Cell culture medium

Positive controls: 4-NQO and B(a)P

Incubation and sampling times: Standardized methodology with 4 hr incubation only

Results

Study validity: The positive and negative controls performed as anticipated. This study is considered adequate for regulatory purposes.

Study outcome: A dose-dependent cytotoxicity was found at all doses of Product Z (51-52% for 100 µg/mL, 27-29% for 200 µg/mL, 22-24% for 300 µg/mL) with or without S9. The positive control 4-NQO had a relative total growth of 84% and B(a)P had a relative growth of 17% after the 4 hr incubation.

A statistically and biologically significant increase in mutant frequencies, large and small colonies at approximately equal values, was found at doses ≥100 µg/mL. The increases were 2.4, 3.2 and 2.9x at 100, 200 and 300 µg/mL without S9 and 3.0, 3.4 and 3.5x with S9, respectively, when compared to controls.

Table 8: 2 Mutagenic activity of pramipexole Product Z in L5178Y cells/tk-locus Nonactivation (4 h exposure) - Summary of results

Test Substance	Culture	Cytotoxicity		Mutant frequencies per 10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: Medium	1	100	100	44	87	141
	2	100	100	49	99	157
	Mean	**100**	**100**	**47**	**93**	**149**
Product Z free base (µg/mL)						
10	1	92	106	56	81	147
	2	78	108	42	64	109
	Mean	**85**	**107**	**49**	**73**	**128**
30	1	80	92	72	152	243
	2	76	100	63	122	200
	Mean	**78**	**96**	**68**	**137**	**222**
100	1	40	49	124	180	344
	2	33	53	114	177	374
	Mean	**37**	**51**	**119**	**179**	**359**
200	1	34	31	174	183	452
	2	27	23	153	256	498
	Mean	**31**	**27**	**164**	**220**	**475**
300	1	22	24	126	225	415
	2	21	20	135	237	435
	Mean	**22**	**22**	**131**	**231**	**425**
Positive Control: 4-NQO 0.1	1	74	84	238	358	833

RS Relative survival
RTG Relative total growth

Table 8: 3 Mutagenic activity of pramipexole Product Z in L5178Y cells/tk- locus With metabolic activation (4 h exposure) - Summary of results

Test Substance	Culture	Cytotoxicity		Mutant frequencies per 10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: Medium	1	100	100	31	89	123
	2	100	100	28	92	126
	Mean	**100**	**100**	**30**	**91**	**125**
Product Z free base (µg/mL)						
10	1	104	87	23	91	119
	2	80	109	26	99	129
	Mean	**92**	**98**	**25**	**95**	**124**
30	1	65	74	59	122	196
	2	72	67	29	128	164
	Mean	**69**	**71**	**44**	**125**	**180**
100	1	53	52	95	211	373
	2	47	51	108	208	368
	Mean	**50**	**52**	**102**	**210**	**371**
200	1	36	23	148	245	473
	2	36	35	107	223	379
	Mean	**36**	**29**	**128**	**234**	**426**
300	1	27	24	111	208	388
	2	29	24	148	260	490
	Mean	**28**	**24**	**130**	**234**	**439**
Positive Control: B(*a*)P 2	1	32	17	361	756	1809

RS Relative survival
RTG Relative total growth

Study title: **Pramipexole Product V (degradation product): Mutagenicity study using micronucleus analysis of rat bone marrow after oral treatment**

Key findings: Under the conditions of this study, Product V (epimer of Product Z) did not elicit cytogenetic damage at single oral gavage doses ≤30 mg/kg.

Study no.: 08B103 or U08-1839-01
Volume and page #: Electronic submission
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany
Date of study initiation: 6/17/08
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: PR2GFK02368PA1 at 93.1% V as HCl

Methods
Strains/species/cell line: Five male Crl:WI (Han) rats/treated group

Doses used in definitive study: 0, 1, 10 or 30 mg/kg as free base equivalent (1.0 g free base= 1.15 g HCL) as a single dose administered by oral gavage at 10 mL/kg

Basis of dose selection: Dose range-finding study where rats died after single doses of 50 or 100 mg/kg

Negative controls: Demineralized water

Positive controls: Cyclophosphamide at 20 mg/kg

Incubation and sampling times: Standardized methodology with sacrifices at 24 hr (all groups) and 48 hr (30 mg/kg dose only)

Results

Study validity: The positive and negative controls performed as anticipated. This study is considered adequate for regulatory purposes.

Study outcome: No adverse clinical signs were observed and all animals survived until designated euthanasia. No significant increases in the mean percentage of polychromatic erythrocytes were noted and there was no significant increase in the micronucleated polychromatic erythrocytes.

Table 8: 1 Micronucleated polychromatic erythrocytes (MNE) and percentages of polychromatic erythrocytes (PCE) in male rats after oral treatment with pramipexole product V - MEAN VALUES

Test substance (mg/kg)	Sampl. time (h)	N	PCE (%)	*p-value*	MNE (%)	*p-value*
Negative vehicle control: Demineralized water	24	5	**42.7**		**0.20**	
Pramipexole product V free base						
1	24	5	**38.2**	*0.12*	**0.22**	*0.80*
10	24	5	**43.6**	*0.71*	**0.24**	*0.55*
30	24	5	**40.5**	*0.39*	**0.19**	*1.00*
	48	5	**37.3**	*0.10*	**0.14**	*0.40*
Positive control: Cyclophosphamide						
20	24	2	**26.0**	*0.05**	**1.03**	*0.05**

* Significantly different from the negative vehicle control ($p \leq 0.05$)

Study title: **Pramipexole Product Z (degradation product): Mutagenicity study using micronucleus analysis of rat bone marrow after oral treatment**

Key findings: Under the conditions of this study, Product Z (degradation product of pramipexole) did not elicit any cytogenetic toxicity in rats treated with single doses of ≤20 mg/kg. Thus this is considered to be a negative outcome study.

Study no.: 08B104 or U08-1838-01
Volume and page #: Electronic submission
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany
Date of study initiation: 6/17/08
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: PR2GFK02359PA1 at 81.8% purity (1.0 g free base= 1.15 g of HCl)

Methods
Strains/species/cell line: Five male Crl:WI (Han) rats

Doses used in definitive study: 0, 1, 10 or 20 mg/kg Product Z (free base equivalent) administered once by oral gavage

Basis of dose selection: Dose range-finding study where rats died at doses ≥30 mg/kg

Negative controls: Demineralized water

Positive controls: Cyclophosphamide at 20 mg/kg

Incubation and sampling times: Standardized methodology with sacrifices at 24 hrs (all doses) and 48 hrs (20 mg/kg dose only)

Results
Study validity: The positive and negative controls performed as anticipated. This study is considered adequate for regulatory purposes.

Study outcome: All animals survived until scheduled sacrifice. Product Z did not elicit any changes in the mean percent of PCEs in rats when compared to the negative controls. There were no dose-dependent increases in micronucleated polychromatic erythrocytes in treated rats when compared to the negative controls.

Table 8: 1 Micronucleated polychromatic erythrocytes (MNE) and percentages of polychromatic erythrocytes (PCE) in male rats after oral treatment with pramipexole Product Z - MEAN VALUES

Test substance (mg/kg)	Sampl. time (h)	N	PCE (%)	*p-value*	MNE (%)	*p-value*
Negative vehicle control: Demineralized water	24	5	**42.7**		**0.20**	
Pramipexole Product Z free base						
1	24	5	**40.1**	*0.36*	**0.21**	*1.00*
10	24	5	**39.2**	*0.25*	**0.25**	*0.50*
20	24	5	**45.8**	*0.45*	**0.18**	*0.83*
	48	5	**39.3**	*0.21*	**0.20**	*1.00*
Positive control: Cyclophosphamide						
20	24	2	**26.0**	*0.05**	**1.03**	*0.05**

* Significantly different from the negative vehicle control ($p \leq 0.05$)

Study title: **Pramipexole Product V (degradation product): Mutagenicity study using the mouse lymphoma (L5178Y) assay**

Key findings: Under the conditions of this study, Product V (degradation product) elicited chromosomal mutations in mouse lymphoma L5178Y cells at doses ≥100 µg/mL. Although the sponsor considered Product V to not be the genotoxicant (they consider catechol to have that role), it remains a positive study with implications for pramipexole.

Study no.: 08B113 or U08-1840-01
Volume and page #: Electronic submission
Conducting laboratory and location: Boehringer Ingelheim Pharma GmbH & Co KG, Biberach an der Riss, Germany
Date of study initiation: 6/24/08
GLP compliance: Yes
QA reports: yes
Drug, lot #, and % purity: PR2GFK02368PA1 at 93.1% purity as HCl
After culturing the Product V for 4 hrs, the sponsor calculated that 2.8, 5.8 and 8.9 µg/mL catechol would have formed after incubation for 1, 2 or 3 hrs, respectively. The findings for the non-GLP stability assay for Product V are as follows:

Table 3.2: 1 Product V (degradation product of pramipexole): Mutagenicity study using the mouse lymphoma (L5178Y) assay - - concentration of Product Z, Product V, catechol, and thiourea in the treatment medium

Substance	Concentration in the treatment medium [%(w/w)]					
	0 h	0.5 h	1 h	2 h	3 h	4 h
Product V	67.1	64.0	60.6	54.3	46.9	39.0
Product Z	0.10	0.13	0.17	0.23	0.29	0.34
Catechol	0.0	1.2	2.8	5.7	8.8	11.9
Thiourea	0.17	1.2	2.4	4.4	6.6	8.7

Methods

Strains/species/cell line: Mouse lymphoma (L5178Y) cells

Doses used in definitive study: 0, 100, 200, 400 or 500 µg/mL

Basis of dose selection: Dose range-finding assay with doses of 50-400 µg/mL with 50% decrease in relative suspension growth found at >200 µg/mL with or without S9 activation

Negative controls: Culture medium

Positive controls: Without S9: 0.1 µg/mL 4-NQO; with S9:

Incubation and sampling times: Standardized methodology

Results

Study validity: The positive and negative controls performed as anticipated. This study is considered adequate for regulatory purposes.

Study outcome: Product V elicited a concentration-dependent increased cytotoxicity with relative growth of 59-62% at 100 µg/mL, 32-34% at 200 µg/mL and 21-26% at 400 µg/mL with or without S9 activation.

A statistically and biologically significant increase in mutant frequencies, both large (higher numbers) and small colonies, was found at doses ≥100 µg/mL. The increases were 3.6, 4.1 and 4.3x at 100, 200 and 400 µg/mL without S9 and 2.8, 3.9 and 3.0x with S9, respectively, when compared to the negative controls. It is unknown why the increases were slightly lower with S9 activation but it contrasts with Study 08-1365-01 where S9 activation elicited a higher mutant frequency distribution.

Table 8: 2 Mutagenic activity of pramipexole Product V in L5178Y cells/tk-locus Nonactivation (4 h exposure) - Summary of results

Test Substance	Culture	Cytotoxicity		Mutant frequencies per 10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: Medium	1	100	100	37	70	114
	2	100	100	30	57	90
	Mean	**100**	**100**	**34**	**64**	**102**
Product V free base (µg/mL)						
10	1	80	116	38	67	109
	2	87	94	40	70	118
	Mean	**84**	**105**	**39**	**69**	**114**
30	1	77	108	61	85	159
	2	110	111	47	109	172
	Mean	**94**	**110**	**54**	**97**	**166**
100	1	42	64	97	200	330
	2	54	59	105	235	401
	Mean	**48**	**62**	**101**	**218**	**366**
200	1	31	38	115	204	382
	2	34	26	128	258	448
	Mean	**33**	**32**	**122**	**231**	**415**
400	1	21	22	139	223	418
	2	16	17	134	250	462
	Mean	**19**	**20**	**137**	**237**	**440**
500	1	19	24	117	197	372
		13	18	118	190	343
	Mean	**16**	**21**	**118**	**194**	**358**
Positive Control: 4-NQO 0.1	1	86	94	183	372	769

RS Relative survival
RTG Relative total growth

Table 8: 3 Mutagenic activity of pramipexole Product V in L5178Y cells/tk-locus With metabolic activation (4 h exposure) - Summary of results

Test Substance	Culture	Cytotoxicity		Mutant frequencies per 10^6 cells		
		RS%	RTG%	small clones	large clones	total
Negative Control: Medium	1	100	100	21	61	85
	2	100	100	29	76	109
	Mean	**100**	**100**	**25**	**69**	**97**
Product V free base (µg/mL)						
10	1	90	89	20	69	91
	2	117	109	34	77	117
	Mean	**104**	**99**	**27**	**73**	**104**
30	1	90	74	39	67	112
	2	113	97	28	75	107
	Mean	**102**	**86**	**34**	**71**	**110**
100	1	64	52	90	144	259
	2	74	66	112	141	285
	Mean	**69**	**59**	**101**	**143**	**272**
200	1	39	29	141	196	407
	2	46	38	115	175	342
	Mean	**43**	**34**	**128**	**186**	**375**
400	1	22	23	96	141	264
	2	37	35	111	159	317
	Mean	**30**	**29**	**104**	**150**	**291**
500	1	34	24	89	156	282
		35	27	86	180	304
	Mean	**35**	**26**	**88**	**168**	**293**
Positive Control: B(*a*)P 2	1	43	35	234	540	1280

RS Relative survival
RTG Relative total growth

Carcinogenicity: None provided

Reproductive toxicology: None provided

Special toxicology: None provided

Genetic Toxicology Consult for Terry Peters
Date: 5/26/09
Reviewer: Anita Bigger
Subject: Genotoxic potential of degradants (Product Z and Product V) of pramipexole drug product (NDA 22-421).

Background

Pramipexole is a non-ergot dopamine D_2 receptor antagonist used for treatment of Parkinson's disease. Currently, pramipexole immediate release (IR) tablets are marketed in the US as MIRAPEX (NDA 20-667). The subject of this application (NDA 22-421) is an extended release (ER) formulation.

Pramipexole itself did not exhibit any genotoxic potential. The subjects of this consult are two potential degradation products (Product V and Product Z) of pramipexole ER tablets that do exhibit genotoxic potential. Currently, Product V and Product Z are controlled under any unspecified degradation products with ≤0.4%. Therefore, the degradants are specified in pramipexole ER tablets as not more than the qualification threshold according to the ICH guideline [ICH Q3B(R)]. However, structural analysis or studies conducted on the IR formulation indicated that the two degradants were genotoxic and the submission contains studies to justify toxicological qualification.

A draft guidance, Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches, addresses limit setting for genotoxic impurities. In the absence of specific information on the carcinogenic potential of a genotoxic impurity, a conservative limit of 1.5 µg/day, based on a threshold of toxicological concern (TTC) risk assessment analysis is considered a safe limit. However, if adequate data are available to allow a compound-specific risk assessment, a different compound-specific limit can be derived.

Sponsor's theory of genotoxicity of Product V and Product Z

Product Z and Product V are epimers with low epimerization potential in solution. However, both degrade in solution in the absence or presence of a rat liver S9 metabolic activation system to catechol, thiourea and propylamine. The sponsor provides studies that support the sponsor's theory that the genotoxicity of Product Z and Product V are due to catechol.

Catechol is a known rat carcinogen causing adenocarcinomas in the glandular stomach after oral administration [Carcinogenic Potency Database Project CPDP)]. It is not carcinogenic in orally-exposed mice but increases the carcinogenic effect of benzo(a)pyrene on the skin in mice when applied together dermally and is therefore, considered to have promoter potential (EPA). The neoplastic changes in rats were strain specific in that the incidence of adenocarcinoma was found in 67, 73 and 77% of Wistar, Lewis and SD rats respectively but in only 10% of WKY rats (Health Canada R08-4391).

EPA has not classified catechol with respect to potential carcinogenicity but IARC has classified catechol as Group 2B, possibly carcinogenic to humans (IARC). Catechol is negative in the Salmonella Ames test for induction of point mutations in bacteria.

Thiourea also induces tumors in male rats in skin (CPDP) and is negative in the Salmonella Ames test. It has been shown to inhibit the mutagenicity of other compounds in mammalian cells in vitro but is also itself weakly genotoxic in mammalian cells in vitro (Ziegler-Skylakakis et al., 1985; Bradley et al., 1982).

Studies on the genotoxicity of Product V and Product Z

1. Product Z (degradation product of pramipexole): Mutagenicity study for chromosomal aberrations in human lymphocytes in vitro (Document/Study No.: 07B127).

A mixture of Product Z and Product V was tested for the ability to induce chromosomal aberrations in human lymphocytes in vitro. Catechol was also tested as a reference compound. Negative and positive controls gave appropriate responses; however, adequate cytotoxicity was not achieved in all arms of the study.

In the presence of metabolic activation, the mixture at the highest dose with acceptable mitotic index (MI) (300 µg/ml, 97% MI) did not induce chromosomal aberrations after 4 hours treatment. However, insufficient acceptable cytotoxicity (50% MI) was not achieved in this arm of the study. Cytotoxicity for the next and highest dose was too high (1000 µg/ml, 32% MI) and the significant induction of chromosomal aberrations that did occur at that dose could have been secondary to the cytotoxicity.

In the absence of metabolic activation, the mixture at the highest dose with acceptable mitotic index (300 µg/ml, 65% MI) did not induce chromosomal aberrations after 4 hours treatment. In the absence of metabolic activation, the mixture at the highest dose with acceptable mitotic index (100 µg/ml, 62% MI) induced a statistically significant but weak increase in aberration frequencies (4.0%) compared to control (0.5%) and historical control range (0 – 2.5%) after 24 hours treatment and 72 hour harvest. Catechol alone did not induce chromosomal aberrations after 4 hours treatment but did induce an increase in the aberration frequencies of 6 and 8% at 1 and 2.5 µg/ml (125% and 113% MI, respectively) after 24 hours treatment and 72 hours harvest. In the case of the aberrations induced by both the mixture and catechol, there were chromatid and chromosome breakage events and acentric fragments but no chromosomal rearrangements, suggesting that the aberrations induced by the mixture were due to the presence of catechol.

Reviewer comment: The mixture of Product Z and Product V did induce chromosomal aberrations in human lymphocytes in vitro. The theory that catechol caused the chromosomal aberrations does not explain the results perfectly. However, given the complexity of the mixture, the presence of thiourea in addition to catechol and the possibility of error in the estimation of amounts of catechol in the mixture, it is a reasonable explanation of the causation of the chromosomal aberrations observed.

2. Product Z (degradation product of pramipexole): Mutagenicity study using the mouse lymphoma (L5178Y) assay (Document/Study No.: 07B134).

This experiment was performed with a mixture of Product Z (7.9%) and Product V (7.6%). The stability of the mixture was determined in treatment medium in the presence of a metabolic activation system consisting of cofactor supplemented rat liver S9 over the course of 4 hours at 37ºC. Catechol was present at 0 hour at 1.7% (w/w). The results demonstrated an approximate 10% (w/w) decrease of Z and V each in concert with a 1% (w/w) increase in catechol, giving a total of approximately 2.5% (w/w) catechol after 4 hours. The results were similar in the absence of the metabolic activation system.

Catechol alone was tested in the microwell version of the mouse lymphoma assay (MLA) for 4 hours at concentrations of 0.1, 0.5, 1, 2.5 and 5.0 µg/ml in the presence and absence of the metabolic activation system. Catechol induced mutations in both the presence and absence of metabolic activation. By adding the Global Evaluation Factor of 126 to the mutant frequency of the negative control to set a cut-off for a negative result, as agreed upon by the International Workshop on Genotoxicity Testing (IWGT), catechol was positive at 2.5 and 5.0 µg/ml in the absence of metabolic activation and at 5.0 µg/ml in the presence of metabolic activation. However, it should be noted that according to IWGT criteria (50 to 170 x 10^6) for a valid assay, the negative control mutant frequency is too high in both the presence (194 x 10^6) and absence (195 x 10^6) of metabolic activation. Never-the-less, catechol was clearly positive above the high background.

The mixture tested positive by IWGT standards in a dose-responsive manner after 4 hours treatment in the absence of metabolic activation at 100, 150, 200, 225 and 250 µg/ml. The assay was valid by IWGT acceptance criteria. The mixture tested positive by IWGT standards after 24 hours treatment in the absence of metabolic activation in one of the duplicates at 100 µg/ml and 13% RTG, within the acceptable cytotoxicity range. The dose range chosen (1, 10, 100, 150 and 200 µg/ml) missed the critical range between 10 and 100 µg/ml that might have shown a dose response at additional acceptable levels of cytotoxicity.

The mixture tested positive by IWGT standards in a dose-responsive manner after 4 hours treatment in the presence of metabolic activation at 100, 250, 300 and 500 µg/ml. The assay was valid by IWGT acceptance criteria.

Based on a comparison of fold-difference over background of positive results induced by the mixture and by catechol alone, the sponsor concluded that the mutant frequencies were within the same order for catechol tested alone and the mixture tested for 4 hours in the absence of metabolic activation. However, the fraction of catechol only appeared to account for about 50% of the total mutant frequency seen with the mixture tested in the presence of metabolic activation. The sponsor stated this may be due to contributions from unknown products or the effect on catechol of the complexity of the mixture containing the metabolic activation system consisting of cofactor supplemented rat liver

S9 fraction. In response to these somewhat puzzling results, the sponsor next conducted experiments on pure Product Z and pure Product V

Reviewer comment: There does seem to be a real difference in the mutant frequencies generated by the mixture in the absence versus the presence of a metabolic activation system. This was not the case with catechol alone where results were similar in the absence or presence of a metabolic activation system. This suggests that catechol cannot account for all the mutagenic activity of the mixture and that Product Z and Product V may be genotoxic also. However, the system is very complex and the presence of thiourea may be an additional complicating factor. The catechol alone experiments do not appear to have been performed at the same time as the mixture, given the difference in mutant frequencies in the negative controls. The fact that the negative controls for the catechol alone are higher than the IWGT recommended range may be a confounding factor also.

3. Pramipexole Product Z (degradation product): Mutagenicity study using the mouse lymphoma (L5178Y) assay (Document/Study No.: 08B082).

The stability of Product Z dissolved in treatment medium was investigated and the amounts of Product Z, Product V, catechol and thiourea determined over the course of 4 hours incubation at 37ºC. At 0 hours, Product Z, Product V, catechol and thiourea were present at 54.3, 0.03, 0.0 and 0.06% (w/w) and at 4 hours, 35.1, 0.26, 8.0 and 6.0% (w/w), respectively. A linear equation was applied to the data and used to calculate that 1.8, 3.8 and 5.7 µg/ml catechol could be formed after incubation of 100 µg/ml Product Z for 1, 2 or 3 hours, respectively.

Product Z was tested in the microwell version of the mouse lymphoma assay in the presence and absence of metabolic activation for 4 hours. Dose-responsive positive results were seen in the presence and absence of metabolic activation at 100, 200 and 300 µg/ml Product Z. The mutant frequencies induced at 100 µg/ml, i.e. 359 x 10^6 in the absence of metabolic activation and 371 x 10^6 in the presence of metabolic activation, are comparable in both cases to the mutant frequencies induced by catechol alone in the previous study (07B134), 447 x 10^6 and 462 x 10^6, respectively, given the continuous release of catechol from Product Z in the present study versus the fixed concentration of catechol in the catechol alone study.

Reviewer comment: In this study there was no difference in mutant frequencies generated by Product Z in the presence or absence of metabolic activation and the mutagenic effects of Product Z can be accounted for by catechol release from Product Z. Although difficult to prove, thiourea or other unknown mutagens do not appear to be contributing to the positive results from Product Z.

4. Pramipexole Product V (degradation product): Mutagenicity study using the mouse lymphoma (L5178Y) assay (Document/Study No.: 08B113).

Reviewer comment: The same approach used for pure Product Z was used to study the mutagenicity of pure Product V in the microwell version of the mouse lymphoma assay. The results were very similar to those found with pure Product Z, with comparable mutant frequencies induced in the presence or absence of metabolic activation. The mutant frequencies induced at 100 µg/ml in the presence (175 x 10^6) and absence (264 x 10^6) were of the same order as those induced by 5 µg/ml catechol tested alone.

5. Risk Assessment based on mutagenicity studies of pure Product Z and pure Product V in the mouse lymphoma assay (Document/Study No.: 08B082 and 08B113).

The sponsor presents a risk assessment for catechol that is based on the worst case scenario, i.e. Product Z and Product V release all the catechol possible given the amounts of Product Z and Product V in the ER and IR drug products. Total catechol per day from Product Z and Product V would be (b) (4) for the IR and ER formulations, respectively. The sponsor further states that catechol is naturally present in food and beverage and that the estimated daily intake of catechol is between 1330 µg (Gold, Slone & Ames R08-4606) and 25,900 to 42,350 µg (for a 50 kg patient with catechol exposure from 518 to 847 µg/kg/day (Health Canada R08-4391). The sponsor concludes that the exposure primarily from food is abou (b) (4) higher than that from pramipexole IR drug product and about (b) (4) higher than that from pramipexole ER drug product.

Reviewer comment: The estimates of daily intake of catechol are based on the amount of catechol in the average daily U.S. consumption of coffee (1330 µg) and the screening assessment for catechol by Health Canada that arrived at a range (25,900 to 42,350 µg) of intake from skimpy data available for catechol in food and beverage. The authors state that the studies available were not from typical food products, were not Canadian-specific and often lacked specific parameters needed to derive realistic estimates. As a result of the paucity of data and taking a conservative approach, the values measured in the various food items were used to represent an entire food group. For example, the concentration of catechol in olive oil was used to represent the entire fats group. The authors state that this likely resulted in an overestimation of the amount of catechol in food. Therefore, the most conservative estimate for the exposure primarily from food is made using the lowest estimate of daily consumption of 1330 µg and in that case, the exposure from food is (b) (4) higher than that from pramipexole IR drug product and about (b) (4) higher than that from pramipexole ER drug product.

I contacted Dan Levy (Levy, personal communication) of CFSAN to see if CFSAN had an estimate of daily consumption of catechol from food and was told that they do not have this information. However, Dr. Levy said that, given the estimate from Health Canada, he would not be concerned to see up to (b) (4) catechol in a dietary supplement.

Another approach to assessing the risk is to calculate safety factors for the level of catechol in pramipexole drug product versus the carcinogenic dose in the 2001 rat carcinogenicity study (Health Canada R08-4391). In the 2 year carcinogenicity study in

male F344 rats catechol was administered in the diet at doses of 33, 65, 141 and 318 mg/kg/day. After a 2 year exposure, adenocarcinomas were observed in the glandular stomach in 3/25 rats at the 318 mg/kg/day dose, while submucosal hyperplasias of the glandular stomach were found at the ≥ 33 mg/kg/day dose. While there is no NOEL for hyperplasias (LOEL of 33 mg/kg/day), 141 mg/kg/day is a NOEL for adenocarcinomas of the glandular stomach. Based on body surface area extrapolation to a human equivalent dose (HED) for a 60 kg human, the HED for the LOEL is 319 mg/day and the HED for the NOEL is 1365 mg/day. Using the more conservative level of (b) (4) (b) (4) catechol from the IR formulation, the safety factor for the LOEL for hyperplasias of the glandular stomach is 5,317 and for the NOEL for adenocarcinomas of the glandular stomach, 22,750. Using the level of (b) (4) for the ER formulation, the safety factor for the LOEL for hyperplasias is 19,938 and the safety factor for the NOEL for adenocarcinomas is 85,313.

Admittedly, the data for both approaches for risk assessment is limited. However, both approaches suggest large safety factors for human exposure to the levels of catechol in pramipexole drug product. Although the most conservative level for genotoxic impurities is 1.5 µg/ml in the absence of studies on the impurity, when specific data on carcinogenic potential are available for an individual risk assessment, a larger amount of the impurity in drug product can be justified.

Recommendation: Since the data for a specific risk assessment for catechol are limited and there is a suggestion that unknown impurities may work synergistically when Product Z and Product V are combined in the mouse lymphoma assay, the sponsor should be encouraged to lower the amounts of Product Z and Product V as low as possible.

References:

Bradley, M.O., Patterson, S. & Zwelling, L.A. (1982) Thiourea prevents cytotoxicity and mutagenicity, but not sister-chromatid exchanges in V79 cells treated with cis-diaminedichloroplatinum (II). Mutation Research 96: 67 -74.

CPDP The Carcinogenic Potency Project (2009) Catechol (CAS 120-80-9) and Thiourea (CAS 62-56-6), http://potency.berkeley.edu/

EPA Catechol (Pyrocatechol) 120-80-9, Technology Transfer Network Air Toxics Web Site (2009), http://www.epa.gov/ttn/atw/hlthef/pyrocate.html

Health Canada (R08-4391) Screening Assessment for the Challenge 1,2-Benzenediol (Catechol) Chemical Abstracts Service Registry Number 120-80-9 (July 2008), PSAP, DSL Program Section 75, pages 1 – 21.

IARC Catechol: Monographs on the evaluation of carcinogenic risk to humans. Re-evaluation of some organic chemicals, hydrozine and hydrogen peroxide. (1999) IARC Monogr Eval Carcinog Risks Hum. 71 (Part 2): 433 – 451.

Levy (personal communication): Dan D. Levy, Ph.D., Division of Dietary Supplement Programs, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration.

Sirsky Gold, L., Slone, T.H. & Ames, B.N. (R08-4606) (1997) Prioritization of possible carcinogenic hazards in food. In: *Food Chemical Risk Analysis* (D. Tennant, ed.), London: Chapman and Hall, pp. 267-295.

Ziegler-Skylakakis, K., Rossberger, S. & Andrae, U. (1985) Thiourea induces DNA repair synthesis in primary rat hepatocyte cultures and gene mutations in V70 Chinese hamster cells. Archives of Toxicology 58: 5 – 9.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: CD 10503, a newly identified degradant from the pramipexole ER tablets, has been shown to form from (b) (4). CD 10503 has been shown to be a definitive bacterial mutagen in the Ames assay. However, given the level of CD 10503 in 4.5 mg pramipexole tablets (specification set at (b)), the exposure would be maximal at (b) (4) This would provide a daily exposure of (b) (4) to a 60 kg person.

It appears that the positive genotoxicity signal elicited by Products Z and V is due, at least in part, to the catechol formed in vitro. CD 10503 (degradation product), Product Z and Product V have been adequately assessed for their genotoxic potential and are present at levels below the thresholds of concern.

Suggested labeling:
None

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22421	ORIG 1		PRAMIPEXOLE DIHYDROCHLORIDE

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

/s/

TERRY S PETERS
08/12/2009

LOIS M FREED
08/19/2009
Please see memo for comments.