CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-436

PHARMACOLOGY REVIEW(S)

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY **1**

PHARMACOLOGY SUMMARY AND CONCLUSIONS 2
LABELING RECOMMENDATIONS 2

II. SAFETY PHARMACOLOGY **3**

SAFETY PHARMACOLOGY SUMMARY AND CONCLUSIONS 8

III. GENERAL TOXICOLOGY **10**

TOXICOLOGY SUMMARY AND CONCLUSIONS 13
LABELING RECOMMENDATION 14

IV. GENETIC TOXICOLOGY **15**

GENETIC TOXICOLOGY SUMMARY AND CONCLUSIONS 29
LABELING RECOMMENDATIONS 32

V. CARCINOGENICITY **34**

LABELING RECOMMENDATIONS 34

VI. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY **35**

LABELING RECOMMENDATIONS 36

VII. SPECIAL TOXICOLOGY **38**

SPECIAL TOXICOLOGY SUMMARY AND CONCLUSIONS 40

VIII. SUMMARY OF LABELING RECOMMENDATIONS **41**

APPENDIX 1 ..... **45**

APPEARS THIS WAY
ON ORIGINAL

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY

The sponsor submitted one pharmacology report: **Effects of repeated administration of aripiprazole and risperidone on serotonin 5-HT_{1A} and 5-HT_{2A} receptors in the rat brain** [Study No. 018472, Report No. 014811].

The purpose of the study was to determine the effects of repeated administration of aripiprazole on $5HT_{1A}$ and $5HT_{2A}$ receptors in rat brain. For comparison, the effects of repeat-dose administration of risperidone on these receptors were also assessed.

Methods: aripiprazole [0, 10, and 30 mg/kg] and risperidone [0.3 mg/kg] were administered to male Wistar rats [6/grp] by gavage [vehicle: 5% gum arabic in 0.9% saline; vol: 5 mL/kg] for 21 days. The HD of aripiprazole was selected on the basis of C_{max}. Previous data indicated that the C_{max} achieved at 30 mg/kg was similar to the C_{max} associated with the maximum recommended human dose [30 mg/day].

Animals were sacrificed 3 days after the last dose of aripiprazole or risperidone. Hippocampus and frontal cortex were dissected from each brain and maintained at -80° C until analyzed. Binding to $5HT_{1A}$ and $5HT_{2A}$ were determined in hippocampus and frontal cortex, respectively.

Results: aripiprazole had no effect on the B_{max} or K_d for either $5HT_{1A}$ or $5HT_{2A}$. However, B_{max} and K_d tended to be increased by aripiprazole in frontal cortex at 10 mg/kg [20%] and at 10 [38%] and 30 mg/kg [54%], respectively. Risperidone significantly increased the B_{max} for $5HT_{1A}$ in hippocampus, but had no significant effect on K_d in hippocampus or on B_{max} or K_d in frontal cortex. The data were summarized in the following sponsor's tables:

Table 1 Effects of repeated administration of aripiprazole and risperidone on serotonin 5-HT_{1A} receptors in the rat hippocampus

	Bmax (fmol/mg protein)	Kd (nM)
Vehicle	578.3 ± 15.9	1.4 ± 0.1
Aripiprazole (10 mg/kg p.o.)	648.0 ± 17.3	1.5 ± 0.1
Aripiprazole (30 mg/kg p.o.)	624.6 ± 33.0	1.5 ± 0.2
Risperidone (0.3 mg/kg p.o.)	697.7 ± 28.7**	1.4 ± 0.1

Table 2 Effects of repeated administration of aripiprazole and risperidone on serotonin 5-HT_{2A} receptors in the rat frontal cortex

	Bmax (fmol/mg protein)	Kd (nM)
Vehicle	686.4 ± 41.6	1.3 ± 0.1
Aripiprazole (10 mg/kg p.o.)	821.9 ± 42.5	1.8 ± 0.2
Aripiprazole (30 mg/kg p.o.)	707.2 ± 121.5	2.0 ± 0.5
Risperidone (0.3 mg/kg p.o.)	756.0 ± 29.0	1.2 ± 0.1

Pharmacology summary and conclusions

The sponsor submitted (1) a pharmacology study assessing the effects of repeated doses of aripiprazole on $5HT_{1A}$ and $5HT_{2A}$ receptors in rat brain and (2) proposed revisions to labeling.

In the pharmacology study, aripiprazole had no significant effect on the B_{max} or K_d for either the $5HT_{1A}$ or $5HT_{2A}$ receptor. Risperidone significantly increased the B_{max} for the $5HT_{1A}$ receptor [in hippocampus], but had no effect on the $5HT_{2A}$ receptor.

The sponsor had one comment on the Pharmacodynamics section of labeling [Revision No. 4]. The sponsor discussed the possible mechanism(s) of action of underlying the antipsychotic effects of aripiprazole, and noted that "Aripiprazole appears to exert its effects through a partial agonist action at D_2 ..and $5\text{-}HT_{1A}$.. receptors and an antagonist action at $5\text{-}HT_{2A}$ receptors".

Reviewer comment: the primary difference between the Agency's and the sponsor's versions of labeling is whether or not language proposing a mechanism of action is included. In general, the mechanism(s) of action of antipsychotic medications is unknown, although there are data that demonstrate a correlation between antipsychotic efficacy and dopamine D_2 antagonism. In addition, perceived differences in the CNS effects between one antipsychotic drug and another may be due to a lack of data rather than an actual difference. Therefore, it is recommended that the Agency's labeling be used.

Labeling recommendations

The following revisions to the sponsor's proposed labeling [version dated 9/18/02] are recommended:

CLINICAL PHARMACOLOGY

Pharmacodynamics

Aripiprazole exhibits high affinity for dopamine D_2 and D_3, serotonin $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptors (K_i values of 0.34, 0.8, 1.7, and 3.4 nM, respectively), moderate affinity for dopamine D_4, serotonin $5\text{-}HT_{2C}$ and $5\text{-}HT_7$, $alpha_1$-adrenergic and histamine H_1 receptors (K_i values of 44, 15, 39, 57, and 61 nM, respectively), and moderate affinity for the serotonin reuptake site (K_i = 98 nM). Aripiprazole has no appreciable affinity for cholinergic muscarinic receptors (IC_{50} > 1000 nM). Aripiprazole functions as a partial agonist at the dopamine D_2 and the serotonin $5\text{-}HT_{1A}$ receptors, and as an antagonist at serotonin $5\text{-}HT_{2A}$ receptor.

The mechanism of action of aripiprazole, as with other drugs having efficacy in schizophrenia, is unknown

Aripiprazole's antagonism of adrenergic receptors may explain the orthostatic hypotension observed with this drug.

II. SAFETY PHARMACOLOGY

A study was conducted in order to investigate ways to antagonize the hypothermia produced by aripiprazole.

Study title: **Prevention of OPC-14597-induced hypothermia after single oral administration to ICR mice under heated condition** [sic] [Study No. 018308, Report No. 014744, study initiation date: 3/25/02].

Methods:

Results: there were no unscheduled deaths. In animals maintained at rm temperature, crouching position, ptosis, cold to the touch, and prone position were observed at both doses. Convulsions were observed in 1/3 LDM [6 hrs postdosing] and 3/3 HDF [2/3 at 6, 12, and 46 hrs postdosing; 3/3 at 24 hrs postdosing]. The convulsions were characterized as "transient" in females. Clinical signs were less severe in animals maintained at elevated temperature. At the LD, crouching position was observed in 1/3 M and ataxia was noted in 2/3 F. At the HD, crouching position and ptosis were observed in all animals, and ataxia was noted in 1/3 F. Mean body wt was reduced in males [12 and 14% at LD and HD, respectively] and females [8 and 14% at LD and HD, respectively] maintained at rm temperature. In animals maintained at elevated temperature, mean body wt tended to be lower in treated males [11-12%, both doses], but was essentially unaffected in treated females.

The body temperature data were summarized in the following sponsor's tables:

Table 2-1
Prevention of OPC-14597-induced Hypothermia after a Single Oral Administration to ICR Mice under Heated Condition
Item : Physiological Examination - Body Temperature
Sex : Male Unit : ℃ Individually housed in steel cages

Dose	Animal No.	PRE	2hr	6hr	12hr	24hr	46hr
A0	00001	36.5	37.4	37.2	37.0	37.3	37.5
	00002	36.6	37.0	36.5	37.4	37.5	37.3
	00003	36.9	37.5	37.5	37.4	37.7	38.2
	Mean	36.67	37.30	37.13	37.27	37.50	37.67
	S.D.	0.21	0.26	0.50	0.23	0.20	0.47
	n	3	3	3	3	3	3
A100	00004	37.2	35.2	34.5	34.5	35.8	36.7
	00005	37.1	32.2	29.9	32.6	35.6	36.4
	00006	36.9	30.1	24.8	23.5	23.7	35.1
	Mean	37.07	32.50*	29.73*	30.27	31.70	36.07
	S.D.	0.15	2.55	4.85	5.92	6.93	0.65
	n	3	3	3	3	3	3
A200	00007	37.0	31.9	27.9	24.3	26.3	35.0
	00008	37.0	31.0	27.7	33.7	35.2	35.6
	00009	37.3	29.0	24.1	23.9	23.2	28.0
	Mean	37.10*	30.63**	26.57*	27.30	28.23	32.87
	S.D.	0.17	1.46	2.14	5.55	6.23	4.23
	n	3	3	3	3	3	3

Table 2-2
Prevention of OPC-14597-induced Hypothermia after a Single Oral Administration to ICR Mice under Heated Condition
Item : Physiological Examination - Body Temperature
Sex : Female Unit : ℃ Individually housed in steel cages

Dose	Animal No.	PRE	2hr	6hr	12hr	24hr	46hr
A0	00019	38.1	37.1	37.9	37.2	36.3	38.4
	00020	37.5	37.0	37.4	37.9	38.0	38.2
	00021	37.8	37.8	37.7	37.8	38.8	38.7
	Mean	37.80	37.30##	37.67##	37.63##	38.37##	38.43
	S.D.	0.30	0.44	0.25	0.38	0.40	0.25
	n	3	3	3	3	3	3
A100	00022	38.3	33.8	33.4	36.7	37.6	38.7
	00023	36.6	29.0	30.8	34.1	37.0	38.2
	00024	37.7	34.9	35.1	35.5	35.4	38.6
	Mean	37.53	32.57	33.10**	35.43*	36.67	38.50
	S.D.	0.88	3.14	2.17@	1.30@	1.14	0.26
	n	3	3	3	3	3	3
A200	00025	37.6	28.7	24.5	25.1	24.9	24.5
	00026	37.5	28.2	24.0	24.2	24.2	26.3
	00027	37.6	31.6	24.1	24.4	24.3	23.7
	Mean	37.57	29.57**	24.20**	24.57**	24.47**	24.83**
	S.D.	0.06	1.95@	0.26@@	0.47@@	0.38@@	1.33@@
	n	3	3	3	3	3	3

Table 2-3
Prevention of OPC-14597-induced Hypothermia after a Single Oral Administration to ICR Mice under Heated Condition
Item : Physiological Examination - Body Temperature
Sex : Male Unit : ℃ Housed by group in a polycarbonate cage on a rubber mat heater

Dose	Animal No.	PRE	2hr	6hr	12hr	24hr	46hr
B0	00010	36.5	37.5	37.9	38.0	37.6	38.0
	00011	36.8	37.8	37.3	37.6	36.8	36.9
	00012	37.3	38.4	36.3	37.4	37.1	37.7
	Mean	36.87	37.90##	37.17##	37.67##	37.17#	37.53
	S.D.	0.40	0.46@@	0.81	0.31	0.40	0.57
	n	3	3	3	3	3	3
B100	00013	36.8	37.0	36.2	36.7	36.9	37.0
	00014	37.2	36.9	37.2	36.5	36.2	37.5
	00015	37.1	36.8	36.2	36.2	35.8	37.7
	Mean	37.03	36.90	36.53	36.47**	36.30	37.40
	S.D.	0.21	0.10	0.58	0.25	0.56	0.36
	n	3	3	3	3	3	3
B200	00016	35.3	34.9	35.6	35.5	35.8	37.1
	00017	36.7	36.1	36.0	35.2	36.1	37.4
	00018	36.3	35.2	35.5	35.6	35.9	37.3
	Mean	35.43	35.40**	35.77	35.43**	35.93*	37.27
	S.D.	0.23	0.62@	0.25@	0.21	0.15@	0.15@
	n	3	3	3	3	3	3

B0 : 0mg/kg heated B100 : 100mg/kg heated B200 : 200mg/kg heated
* : P<0.05 , ** : P<0.01 : Significant difference from control (B0)
# : P<0.05 , ## : P<0.01 : Significant dose dependency
@ : P<0.05 , @@ : P<0.01 : Significant difference from the pre-value

Table 2-4
Prevention of OPC-14597-Induced Hypothermia after a Single Oral Administration to ICR Mice under Heated Condition
Item : Physiological Examination - Body Temperature
Sex : Female Unit : ℃ Housed by group in a polycarbonate cage on a rubber mat heater

Dose	Animal No.	PRE	2hr	6hr	12hr	24hr	48hr
B0	00028	37.4	38.5	38.0	37.6	37.0	37.9
	00029	37.5	38.7	38.1	38.2	37.8	38.1
	00030	37.4	38.0	38.5	38.1	37.6	37.6
	Mean	37.43	38.73●●	38.20●●	37.97●	37.47	37.87
	S.D.	0.06	0.25●	0.26●	0.32	0.42	0.25
	n	3	3	3	3	3	3
B100	00031	37.8	38.0	37.5	38.0	38.2	38.8
	00032	37.3	37.4	37.6	38.2	37.3	38.9
	00033	37.7	38.0	37.2	38.0	38.1	38.8
	Mean	37.60	37.80●	37.43●●	38.07	37.87	38.83●
	S.D.	0.26	0.35	0.21	0.12	0.49	0.06●
	n	3	3	3	3	3	3
B200	00034	37.5	35.8	35.5	36.5	37.4	37.8
	00035	37.7	36.1	35.4	36.4	37.6	37.9
	00036	37.9	35.1	35.2	37.3	37.3	38.8
	Mean	37.70	35.67●●	35.37●●	36.73●●	37.43	38.17
	S.D.	0.20	0.51●	0.15●●	0.49●	0.15	0.55
	n	3	3	3	3	3	3

B0 : 0mg/kg heated B100 : 100mg/kg heated B200 : 200mg/kg heated
● : P<0.05 , ●● : P<0.01 : Significant difference from control (B0)
● : P<0.05 , ●● : P<0.01 : Significant dose dependency
● : P<0.05 , ●● : P<0.01 : Significant difference from the pre-value

Study title: **Effects of BMS-337039, BMS-337039 metabolites, and comparator antipsychotics on HERG/I_{Kr} currents and rabbit Purkinje fiber action potentials** [no study no. provided; study period: 6/17/02-7/31/02, Bristol-Myers Squibb Pharmaceutical Research Institute, NJ, **non-GLP**]

Methods:

Results: all compounds, with the exception of OPC-3373, produced concentration-dependent inhibition of HERG. The data are summarized in the following sponsor's table:

Table 1 Comparative effects of aripiprazole (n=4), OPC-14857 (n=4), OPC-3373 (n=4), haloperidol (n=4), risperidone (n=4), ziprasidone (n=5), quetiapine (n=4), olanzapine, (n=4) and cisapride (n=4) on HERG currents expressed in HEK293 cells. Values are mean±SEM.

	% inhibition at								
Drug	**0.003**	**0.01**	**0.03**	**0.1**	**0.3**	**1**	**3**	**10 μM**	**IC_{50}μM**
haloperidol	13.6±1.8	28.6±4.6	55.9±3.7	81.5±3.2	91.7±1.2				0.027
risperidone		7.1±3.6	15.2±2.9	30.7±2.5	58.8±2.6	82.9±2.7			0.226
ziprasidone	7.0±3.1	11.3±3.7	22.1±3.7	50.4±3.3	72.8±3.0	92.1±0.7			0.100
quetiapine					5.2±1.4	16.5±4.3	41.0±2.4	63.9±2.4	7.6
olanzapine					11.0±3.7	16.4±3.7	32.4±5.1	54.7±5.0	7.3
aripiprazole			8.8±0.5	25.3±3.8	53.4±4.6	81.0±2.9	93.7±1.1		0.263
OPC-3373					2.2±1.3	1.9±2.1	2.6±2.1	4.8±2.3	inact.
OPC-14857			7.0±1.8	21.9±2.8	52.4±3.1	79.2±1.8	94.6±1.0		0.289
cisapride		22.0±1.2	52.4±3.1	82.1±4.1	94.2±0.9				0.028

The order of potency, from greatest to least was as follows: cisapride = haloperidol > ziprasidone > risperidone > aripiprazole ≈ OPC-14857 >>olanzapine ≈ quetiapine.

In rabbit Purkinje fibers, haloperidol, risperidone, and cisapride, and to a lesser extent, ziprasidone and olanzapine, produced concentration-dependent increases in APD_{50} and/or APD_{90}. Maximum increases in APD_{90} were 63, 86, 110, 27, and 20%, respectively. Aripiprazole, OPC-14857, OPC-3373, and quetiapine did not notably affect APD. [The data were summarized in the sponsor's table provided on the following page.] As illustrated in the following sponsor's figure, haloperidol and risperidone were associated with EADs at the HCs tested.

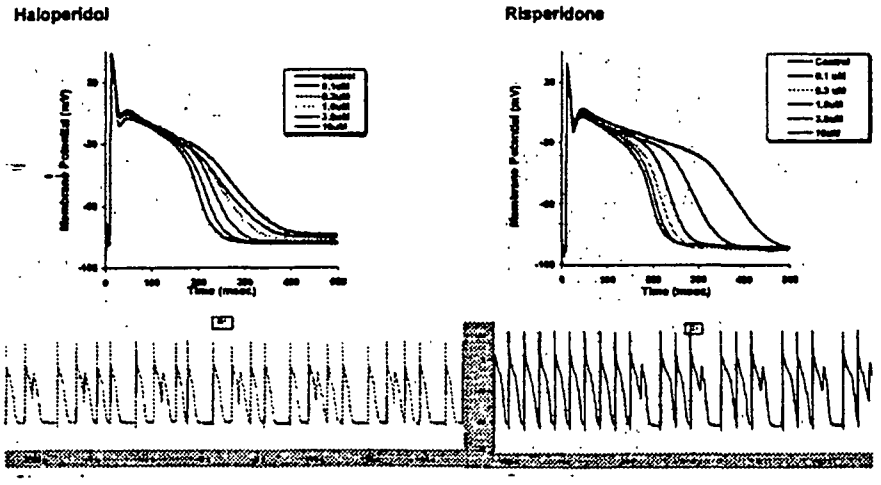

Table 4 Comparative effects of risperidone, haloperidol, quetiapine, olanzapine, ziprasidone, aripiprazole (n=3), OPC-14857 (n=3), OPC-3373 (n=3), and cisapride on action potential duration at 50% and 90% repolarization (APD50 and APD90) recorded in rabbit Purkinje fibers stimulated at the frequency of 1 Hz.

APD_{50} = time to 50% action potential repolarization and APD_{90} = time to 90% action potential repolarization

	Conc. (μM)	0	0.1	0.3	1.0	3.0	10.0
Risperidone	APD50 (ms)						
n=4	Mean±sem	172±9	192±13	211±16	249±31	286±36	279±9
	% Δ	0±0	12±2	22±3	43±11	64±13	70±13
	APD90 (ms)						
	Mean±sem	231±12	259±17	288±22	348±50	430±60	429±2
	% Δ	0±0	12±3	24±4	49±14	84±15	95±5
Haloperidol	APD50 (ms)						
n=3	Mean±sem	155±4	176±10	203±27	217±27	171±15	164±5
	% Δ	0±0	13±4	30±13	39±14	9±6	6±5
	APD90 (ms)						
	Mean±sem	232±7	263±15	315±32	357±37	369±33	379±31
	% Δ	0±0	13±3	36±12	54±14	59±12	64±11
Olanzapine	APD50 (ms)						
n=3	Mean±sem	139±4	144±6	150±6	156±6	157±7	151±13
	% Δ	0±0	4±2	8±2	12±2	13±2	8±7
	APD90 (ms)						
	Mean±sem	227±8	229±7	237±8	246±7	258±8	273±9
	% Δ	0±0	1±1	4±0	8±3	14±3	21±2
Quetiapine	APD50 (ms)						
n=3	Mean±sem	169±4	174±4	184±8	187±6	189±7	171±1
	% Δ	0±0	4±3	9±7	11±6	12±6	0±5
	APD90 (ms)						
	Mean±sem	246±15	249±13	257±13	259±13	261±16	263±22
	% Δ	0±0	1±1	4±2	6±3	6±0	3±0
Ziprasidone	APD50 (ms)						
n=3	Mean±sem	154±28	159±25	160±26	173±25	189±22	195±29
	% Δ	0±0	5±4	5±3	14±4	27±11	29±7
	APD90 (ms)						
	Mean±sem	228±19	230±18	237±18	247±21	270±20	290±29
	% Δ	0±0	1±1	4±2	9±0	19±2	27±3
Aripiprazole	APD50 (ms)						
n=3	Mean±sem	168±26	172±25	176±25	184±25	183±24	184±26
	% Δ	0±0	3±2	5±2	10±2	10±4	10±3
	APD90 (ms)						
	Mean±sem	250±17	249±16	253±14	257±15	257±16	260±17
	% Δ	0±0	0±1	1±1	3±1	3±1	4±0

APD_{50} = time to 50% action potential repolarization and APD_{90} = time to 90% action potential repolarization (continued)

	Conc. (μM)	0	0.1	0.3	1.0	3.0	10.0
OPC-14857	APD50 (ms)						
n=3	Mean±sem	164±16	167±17	167±14	173±20	170±15	172±15
	% Δ	0±0	2±1	2±3	5±3	4±2	5±3
	APD90 (ms)						
	Mean±sem	225±16	225±16	227±14	230±16	229±13	234±13
	% Δ	0±0	0±0	1±1	2±1	2±2	4±2
OPC-3373	APD50 (ms)						
n=3	Mean±sem	188±3	189±6	194±5	200±7	194±12	200±8
	% Δ	0±0	1±2	3±2	6±3	3±6	7±4
	APD90 (ms)						
	Mean±sem	264±19	263±18	267±18	271±17	272±19	274±20
	% Δ	0±0	0±0	1±1	3±1	3±2	4±3
Cisapride	APD50 (ms)						
n=3	Mean±sem	143±4	195±20	214±20	193±13	190±11	
	% Δ	0±0	38±18	51±12	36±13	29±11	
	APD90 (ms)						
	Mean±sem	221±14	283±40	401±52	466±58	435±15	
	% Δ	0±0	27±11	77±13	106±18	110±14	

Risperidone, haloperidol, quetiapine, OPC-14857, OPC-3373, and cisapride decreased maximum upstroke velocity [29, 43, 30, 31, 16, and 18%, respectively]; aripiprazole, ziprasidone, and olanzapine had no notable effect on this parameter.

None of the drugs tested had notable effects on RMP or overshoot.

Safety pharmacology summary and conclusions

The sponsor conducted two safety pharmacology studies, one to investigate the hypothermic effects of aripiprazole and one to assess the cardiovascular effects of aripiprazole in the *in vitro* HERG and rabbit Purkinje fiber assays.

Hypothermia: aripiprazole was administered to CD-1 mice at acute doses of 0, 100, and 200 mg/kg. Animals were housed either at rm temperature [housed individually] or under heated [grp housed] conditions. Rectal temperature was recorded up to 46 hrs postdosing.

In animals maintained at rm temperature, mean rectal temperature was significant decreased at both doses. At 100 mg/kg, mean rectal temperature was significantly decreased in males at 2 and 6 hrs postdosing and in females at 6 and 12 hrs postdosing. In individual animals, lowest rectal temperatures were recorded at 6 and 12 hrs in males [23.5-34.5° C] and at 2 and 6 hrs in females [29.0-34.9° C]. Rectal temperature fell below 30° C in 2/3 M and 1/3 F. At 200 mg/kg, mean rectal temperature was significantly decreased at 2 and 6 hrs postdosing in males and at 2, 6, 12, 24, and 46 hrs in females. In individual animals, lowest rectal temperatures were recorded at 6-24 hrs postdosing [23.2-27.7° C] in males and at 6 and 46 hrs postdosing [24.0-24.5° C] in females. Rectal temperature fell below 30° C in all HDM and HDF. By the end of the 46-hr observation period, rectal temperature was still <30° C in 1/3 HDM and 3/3 HDF.

In animals maintained under heated conditions, the hypothermic effects of aripiprazole were significantly attenuated. Although there were significant decreases in mean rectal temperature relative to C at both doses, mean rectal temperature remained above 36° C at 100 mg/kg and above 35° C at 200 mg/kg in both males and females. By 46 hrs postdosing, mean rectal temperature was similar or slightly higher in HD animals compared to Cs.

Cardiovascular effects: the sponsor assessed the effects of aripiprazole, metabolites, OPC-14857 and OPC-3373, and various other antipsychotic drugs in the *in vitro* HERG and rabbit Purkinje fiber assays. Cisapride was used as a positive control in both assays. All compounds, with the exception of OPC-3373, produced concentration-dependent inhibition of HERG; the IC_{50}'s for aripiprazole and OPC-14857 were 0.263 and 0.289 μM, respectively [IC_{50} for cisapride = 0.028 μM].

In rabbit Purkinje fibers, haloperidol, risperidone, and cisapride, and to a lesser extent, ziprasidone and olanzapine, produced concentration-dependent increases in APD_{50} and/or APD_{90}. In addition, EADs were observed following treatment with haloperidol and risperidone. Aripiprazole, its metabolites [OPC-14857, OPC-3373], and quetiapine had no notable effect on APD.

Aripiprazole, ziprasidone, and olanzapine had no notable effect on maximum upstroke velocity, whereas metabolites, OPC-14857 and OPC-3373, and the other compounds tested produced decreases in this parameter.

The data from the HERG assay would suggest that aripiprazole and one [OPC-14857] of the two metabolites tested has the potential to cause QT prolongation *in vivo*. The fact that neither compound

prolonged the APD in the rabbit Purkinje fiber assay does not necessarily diminish the potential for concern. However, to this reviewer's knowledge, drug-induced QT prolongation has not been observed in humans at clinically relevant doses of aripiprazole.

APPEARS THIS WAY
ON ORIGINAL

III. GENERAL TOXICOLOGY

1. Study title: Twenty-eight-day repeated-dose oral toxicity study of 2,3-DCPP in rats.

Study no: 018332
Volume #: 103.2
Conducting laboratory and location:
Date of study initiation:
GLP: conducted according to the "OECD Principles of" GLP
QA report:Y
Drug, lot #, radiolabel, and % purity:2,3-DCPP, lot no. 400000003, purity stated to be
Formulation/vehicle: solution/purified water; solutions were prepared weekly. Stability and homogeneity of the dosing solution was assessed; DCPP was found to be stable in solution for 7 days, and solutions were found to be homogenous.

Methods
Dosing

Species/strain: CD(SD) rat [
housing: 5/cage
#/sex/group or time point (main study): 6/sex/grp
Satellite groups used for toxicokinetics or recovery: 0, 10, and 30 mg/kg recovery grps [6/sex/grp].
Age: 5 wks
Body Wt: 145.7-176.8 gm for males, 119.4-149.2 gm for females
Doses in administered units: 0, 3, 10, 30 mg/kg
Route, form, volume, and infusion rate: p.o., gavage, 10 mL/kg.

Observations and times

Clinical signs: clinical signs were recorded three times per day [prior to, during, and after dosing] during the dosing period in all animals. In recovery animals, clinical signs were recorded daily during the recovery period.
Body weights: body wts were recorded for all animals prior to start of dosing, on Days 1, 3, 8, 12, 17, 21, 26, and 28 of dosing, and on Days 1, 5, 10, and 14 of recovery. Body wts were also recorded at necropsy.
Food consumption: food consumption was recorded prior to the start of dosing, on Days 3, 8, 15, 22, and 28 of dosing, and on Days 4, 8, and 14 of recovery.
Ophthalmoscopy: n/a
EKG: n/a
Hematology: blood samples were collected at the end of the dosing and recovery periods for analysis of the following parameters: rbc ct, wbc ct [total, differential], hgb, hct, MCV, MCH, MCHC, platelet ct, reticulocyte ct, PT, APTT.
Clinical chemistry: blood samples were collected according to the same schedule used for analysis of hematology; the following parameters were assessed: ALT, AST, alkaline phosphatase, cholinesterase, γ-GTP, total cholesterol, TG, glucose, total protein, albumin, A/G ratio, BUN, creatinine, total bilirubin, Ca, P_i, Na, K, Cl.
Urinalysis: urine samples were collected over a 16-hr period on Day 28 of dosing and on Day 14 of recovery in all survivors. The following parameters were assessed: volume, color, pH, protein, ketone bodies, bilirubin, occult blood, glucose, urobilinogen.
Gross pathology: a necropsy was performed on all animals.
Organs weights: the following organ wts were recorded in all animals: liver, kidneys, testes, ovaries, brain, spleen, adrenals.
Histopathology: the following tissues were examined microscopically:

C grp: stomach, intestine ["duodenum to rectum"], pancreas, liver, heart, kidneys, testes, brain, spinal cord, sciatic nerve, spleen, adrenals.
C grp [recovery]: testes
MD grp [recovery]: testes
HD grp: stomach, intestine ["duodenum to rectum"], pancreas, liver, heart, kidneys, testes, brain, spinal cord, sciatic nerve, spleen, adrenals.
HD grp [recovery]: testes

All tissues, except for testes and epididymides, were fixed in 10% neutral buffered formalin; testes and epididymides were fixed in Bouin's solution.

Toxicokinetics: n/a

Results

Mortality: two HDM died prematurely [#33 (Day 19), #34 (Days 21)]. Salivation, miosis, irritability, and exophthalmos were observed in both animals. Animal #34 also exhibited tonic convulsions [nos], paleness, and piloerection. It was noted that salivation was noted "almost continuously...immediately after dosing on day 3 to the day of death".

Clinical signs: clinical signs were not observed in LD animals. Salivation, miosis, exophthalmos, and irritability were observed in all MD and HD animals [except that irritability was observed in 5/6 MDF]. Tremors were also noted in HDM. No drug-related findings were observed during the recovery period.

Body weights: mean body wt was significantly reduced in MDM and HDM [9 and 18%, respectively, on Day 28 (compared to CM)]; the effect was observed on Days 17-28 at the MD and on Days 3-28 at the HD. Body wt was not affected in females. Mean body wt remained reduced [compared to CM-R] during the recovery period; however, some normalization was observed [14% decrease on Days 1-5 of recovery; 10-8% on Days 10-14].

Food consumption: mean food intake was significantly decreased in HDM throughout the dosing period [11-29%], and sporadically in MDM [8-11% on Days 3 and 28]. In females, food intake was transiently reduced at the HD [22%, Day 3]. Food intake was similar among grps during the recovery period.

Hematology: there were no clear drug-related effects.

Clinical chemistry: the only findings of note were small increases in ALT and AST [31 and 24%, respectively] and a decrease in serum Ca [8%] in HDF, and an increase in γ-GTP [100%] and a decrease in P_i [11%] in HDM. [ALT and AST were particularly increased in 2 HDF (104 and 110 IU/L vs 63-84 IU/L in CF); γ-GTP was higher in all surviving HDM compared to CM (0.8-1.2 UI/L vs 0.4-0.7 IU/L).] These effects were not observed in recovery animals. Total bilirubin was increased in HDM-R [27%]; however, ALT and AST were not increased in a dose-related manner.

Urinalysis: no apparent drug-related effects.

Organ weights: in males, absolute, but not relative, liver wt was decreased at the MD and HD [18 and 23%, respectively]; relative testis and brain wts were increased at the MD and HD [testis: 17-24%; brain: 15-29%]. No findings were observed in recovery animals.

Gross pathology: there were no clear drug-related findings. There were no findings in the 2 HDM that died prematurely.

Histopathology: there were no apparent drug-related findings. Testicular findings [diffuse atrophy of the seminiferous tubules, Leydig cell hyperplasia] were detected in 1/6 HDM-R; these findings correlated with "small testis" detected upon macroscopic examination.

2. Study title: BMS-337039: 39-week oral gavage toxicity study in cynomolgus monkeys. Final Report Amendment No. 1.

Amendment No. 1 provided a cardiology report with tabulated ECG data for the 39-wk oral toxicity study previously submitted [and reviewed in the original NDA review].

Methods: ECG parameters were recorded for all animals, using 10-lead ECGs [I, II, III, aVR, aVL, aVL, V1, V2, V3, V4; 50 mm/sec], prior to start of dosing and following the daily dose [≈2-4 hrs postdosing] during Wks 13, 26, and 39. The following parameters were recorded using Lead II: hr, P-wave duration, P-R, QRS, QT, and QT_c, P, R, and T wave amplitudes, and ST. QT_c was calculated using the method of Spence *et al.* [Spence S. *et al. Toxicological Sci* 45:247-258, 1998]: $QT_c = QT - 0.087*([60/hr]-1)$.

Results: there was no apparent drug-related effect on any of the ECG parameters.

3. Study title: BMS-337039: 39-week oral gavage toxicity study in cynomolgus monkeys: Final Report Amendment No. 2 [Study No. 99354, , amendment approval signed 7/26/02].

Amendment No. 2 provided the results of qualitative analysis of liver concretions [hepatoliths], as requested by the sponsor. In addition, "...errors in sample identification in the text of the Gallsand, Gallstone, and Bile Analytical Reports..." were corrected.

Methods:

Results: according to the text of the revised study report, the analysis of the slides from Animals #I06748 and I06719 indicated "...morphological features and an elemental composition that were similar to gallsand and gallstones (i.e., structures composed of the sulfate conjugates of hydroxy metabolites of BMS-337039)".

The sponsor also included the report, "Intrahepatic concretion (hepatoliths) analysis, BMS Study No. 99354:BMS-337039: Thirty-nine week oral gavage toxicity study in cynomolgus monkeys", from The analyses conducted by were not under GLP.

Various analytic techniques were used to examine the formalin-fixed liver sections [e.g., polarized light microscopy, fluorescence microscopy]. However, attempts to examine the concretions *in situ* were unsuccessful. Therefore, the concretions were removed from the tissue samples and examined by

EDS analysis of H & E-stained samples from Animal #I06748 confirmed that the elemental content of the concretions were "..consistent with the composition of the sulfate conjugates of hydroxy aripiprazole (DM-1451, BMS-37042), or dehydro hydroxy aripiprazole (DM-1460, BMS-511426), previously identified as major drug-related constituents of gallsand and gallstones". The PAS-stained samples could not be analyzed.

EDS analysis of H & E- and PAS-stained samples from Animal #I06719 was uninformative.

Toxicology summary and conclusions

The sponsor conducted a 28-day toxicity study of metabolite, 2,3-DCPP, in Sprague-Dawley rats. In the original NDA database, 2,3-DCPP was reported to be present in rat and monkey plasma and in human urine. In the 28-day study, 2,3-DCPP [DCPP] was administered at doses of 0, 3, 10, and 30 mg/kg p.o. Observations consisted of the following: clinical signs, body wt, food consumption, hematology, clinical chemistry, urinalysis, and terminal studies [gross pathology, organ wts, selected histopathology]. Additional animals [C, MD, HD] were observed during a 2-wk recovery period.

There were 2 unscheduled deaths at the HD, both in males [Days 19 and 21 of dosing]. Clinical signs observed prior to death included salivation, miosis, irritability, exophthalmos, and convulsions [1 animal]. In survivors, clinical signs were observed at all but the LD; these included salivation, miosis, irritability, exophthalmos, and tremors [HDM only]. No drug-related clinical signs were evident in recovery animals. Mean body wt was significantly reduced in MD and HD males, but was not affected in females. Body wt remained reduced in drug-treated males during the recovery period; however, some normalization was noted. Food intake was consistently reduced in HDM, sporadically reduced in MDM, and transiently reduced [Day 3 of dosing] in HDF. No effect on food intake was observed during the recovery period. There were no clear drug-related effects on hematology or urinalysis parameters. Drug-related effects were noted on selected clinical pathology parameters [ALT, AST, and Ca in HDF; Pi and γ-GTP in HDM]; however, no parameter was affected in both males and females. These parameters were not affected in recovery animals. No drug-related gross lesions were detected either in survivors or in the 2 HDM that died prematurely. Absolute, but not relative, liver wt was reduced in drug-treated males, consistent with effects on body wt. Relative, but not absolute, wts of testes and brain were increased in drug-treated males, presumably reflecting a sparing of these organs in spite of decreases in body wt gain. There were no apparent drug-related microscopic lesions. Diffuse atrophy of the seminiferous tubules and Leydig cell hyperplasia were detected in 1 HDM sacrificed at the end of the recovery period.

A major target organ for toxicity appears to be the NS. However, the study was not an adequate assessment of the general toxicity of 2,3-DCPP since only selected tissues were examined microscopically.

The information provided in the toxicology reports submitted by the sponsor have no impact on the approvability of the NDA or on labeling.

Labeling: the sponsor has proposed removing the animal toxicology information from the PRECAUTIONS section and has proposed revisions to the ANIMAL TOXICOLOGY section of labeling. In comments supporting the removal of the information from the Precautions section [Revision No. 18], the sponsor noted that findings of retinal degeneration observed with ropinirole [REQUIP™] and pramipexole [MIRAPEX™] appeared to be included in the PRECAUTIONS section due to the lack of a safety margin for humans. In contrast, citalopram [Celexa™] produces retinal degeneration at "...a dose representing a high multiples (13 times) of the MRHD...whereas the no-effect dose for this effect was approximately 4 times the MRHD on a mg/m^2 basis". For citalopram, the retinal findings are included only in the ANIMAL TOXICOLOGY section of labeling.

A search of the PDR for reports of "retinal degeneration" in animals indicated no consensus on the placement of the discussion of the animal findings. For example, optic nerve degeneration observed in animals treated with Mevacor [lovastatin] was described in the PRECAUTIONS section only, even though the low-effect level was associated with "mean plasma drug levels about 30 times higher than the mean plasma drug level in humans" at the MRHD. Similarly, ocular/optic nerve findings in animals treated with Zocor [simvastatin] were described in the PRECAUTIONS section only; findings were

observed at doses associated with plasma AUCs 5-25 times [depending on species, specific finding, and duration of study] that achieved in humans at the MRHD.

Retinal degeneration and corneal opacities observed in animals treated with Zanaflex [tizanidine] were presented in the PRECAUTIONS sections only; these findings were noted at doses similar to the MRHD on a mg/m^2 basis.

Ocular lesions observed in animals treated with Lariam [mefloquine] were reported both in the PRECAUTIONS and ANIMAL TOXICOLOGY sections. No interspecies comparisons were made; however, based on the recommended dose for prophylactic use, it would appear that the findings in animals were detected at doses within the clinical dose range on a mg/m^2 basis.

Therefore, for even this limited search, there is no uniform treatment of animal data in labeling. However, it would appear that, in this division, there is a tendency to include retinal findings in the PRECAUTIONS section in cases where there is little or no safety margin. Therefore, the sponsor's revision appears reasonable.

Labeling recommendation

The following changes to the sponsor's proposed labeling [version submitted 9/18/02] are recommended:

" **ANIMAL TOXICOLOGY**

Aripiprazole produced retinal degeneration in albino rats in a 26-week chronic toxicity study at a dose of 60 mg/kg and in a 2-year carcinogenicity study at doses of 40 and 60 mg/kg. The 40- and 60-mg/kg doses are13 and 19 times the maximum recommended human dose based on mg/m^2 and 7 to 14 times human exposure at MRHD based on AUC. Evaluation of the retinas of albino mice and of monkeys did not reveal evidence of retinal degeneration. Additional studies to further evaluate the mechanism have not been performed. **The relevance of this finding to human risk is unknown.**

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ON ORIGINAL

IV. GENETIC TOXICOLOGY

1. Study title: Ames reverse-mutation study in Salmonella and *Escherichia coli.*

Study no: DS02115
Volume #: 103.1
Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute, Syracuse, NY
Date of study initiation: 6/17/02
GLP compliance: Y
QA report: Y
Drug, lot #, radiolabel, and % purity: OPC-3373 [aka BMS-337047], lot no. 01F12P, purity

Formulation/vehicle: solution/DMSO

Methods:

Strains/species/cell line: *S. typhimurium* TA98, TA100, TA1535, TA1537, *E. coli* WP2uvrA
Dose selection criteria:
Basis of dose selection: dose-range finding study
Range finding studies: a dose range-finding study was conducted in all tester strains using concentrations of 50-5000 µg/plate. Cultures were assessed in duplicate.

Analysis:

Results

Range-finding study: no significant cytotoxicity was observed in the range-finding study. At 5000 µg/plate, minimal reduction in background lawn was noted with all *S. typhimurium* strains in the absence of S9, and TA98, TA1535, and TA1537 in the presence of S9. A moderate decrease in background lawn was observed with TA100 in the presence of S9. No effect on background lawn was noted with WP2uvrA, either with or without S9. No increases in revertants were observed with any tester strain, with or without S9.

Definitive study: minimal reduction in background lawn was observed at the HC with TA98, TA100, TA1535, and WP2uvrA, both with and without S9. No cytotoxicity was observed with TA1537, either with or without S9. No increases in mean revertants were observed with any tester strain, with or without S9. Positive controls produced increases in mean revertants consistent with positive responses.

2. Study title: Ames reverse-mutation study in Salmonella and *Escherichia coli.*

Study no: DS02116
Volume #: 103.1
Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute, Syracuse, NY.
Date of study initiation: 7/12/02
GLP: Y
QA report: Y
Drug, lot #, radiolabel, and % purity: OPC-14857, lot no. 02E14P, purity =

Formulation/vehicle: solution/DMSO

Methods:
Strains/species/cell line: TA98, TA100, TA1535, TA1537, *E. coli* WP2uvrA
Dose selection criteria:
Basis of dose selection: dose-range finding study
Range finding studies: concentrations of 1.5-300 µg/plate were tested on all tester strains

Results

Range-finding study: marked reduction- to-"annihilation" of background lawn was observed with all tester strains at 300 µg/plate. Moderate-to marked reduction of background lawn was observed with all

tester strains, except for WP2uvrA, at 100 µg/plate. At concentrations up to 100 µg/plate, no increases in mean revertants were observed with any tester strain, either with or without S9.

Definitive study: cytotoxicity [i.e., moderate-to marked decrease in background lawn] was observed with all *S. typhimurium* strains [±S9] at 64 µg/plate, and with WP2uvrA at 256 µg/plate. There were no increases in mean revertants with any tester strain, with or without S9. Positive controls produced increases in mean revertants consistent with positive responses.

3. Study title: Micronucleus test of OPC-14597 in peripheral blood and bone marrow erythrocytes after single oral administration to ICR mice with or without heating.

Study no: 018330
Volume #: 103.1
Conducting laboratory and location: Otsuka Pharmaceutical Co., Ltd.
Date of study initiation: 5/16/02
GLP:Y
QA report: Y
Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. C98G92(2)M, purity

Formulation/vehicle: suspension/5% gum arabic

Methods:

Strains/species/cell line: CD-1(ICR) mice [8-9 wks of age]
Housing: Grps A and B: 1/cage; Grps C, D, F: 3/cage, Grps E2: 3/cage.
Dose selection criteria:
Basis of dose selection: previous studies.
Test agent stability: stated to be stable and homogenous in suspension for 24 hrs at room temperature, protected from light. [No data provided to document stability or homogeneity included in report.]
Metabolic activation system: n/a
Controls
Negative controls: vehicle
Positive controls: Mitomycin C
Comments: additional animals were treated with reserpine.
Exposure conditions
Incubation and sampling times: cf. summary table.
Doses used in definitive study: cf. summary table.
Study design: the experimental design was summarized in the sponsor's table below [day of administration designated Day 1]. Observations consisted of clinical signs [prior to dosing, and at 2, 4, 8, 12, 24, and 46 hrs postdosing; all Grp A, B, C, and D animals], body wt [prior to dosing, Days 1 and 3], body temperature [rectum; prior to dosing and at 2, 4, 8, 12, 24, and 46 hrs postdosing; Grps A, B, C, D]. No necropsy was performed. Blood samples were collected [from the posterior vena cava] for TK analysis [parent compound, OPC-14857, DM-1451, DM-1452, DCPP, OPC-3373, using for all compounds)] at 1, 2, 4, 8, and 24 hrs postdosing [Grp F200, 3/sex], and at 48 hrs postdosing [Grp C200, 6/sex].

1) Experimental Groups

Individually housed in stainless steel cages

Group#1	Dose (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)	Time#2 (h)	Number of animals (Animal nos.) Male	Female	ID card color
A0	0	0	10	48	6 (00001–006)	6 (00070–075)	White
A100	100	10	10	48	6 (00007–012)	6 (00076–081)	Yellow
A200	200	20	10	48	6 (00013–018)	6 (00082–087)	Orange
B200	200	20	10	48	6 (00019–024)	0	Pink

Housed by group in a polycarbonate cage with chips on a rubber mat heater (Mat nos. 1 and 2)

Group#1	Dose (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)	Time#2 (h)	Number of animals (Animal nos.) Male	Female	ID card color
C0	0	0	10	48	6 (00025–030)	6 (00088–093)	White
C100	100	10	10	48	6 (00031–036)	6 (00094–099)	Yellow
C200	200	20	10	48	6 (00037–042)	6 (00100–105)	Orange
D200	200	20	10	48	6 (00043–048)	0	Pink

Housed by group in a disposable cage with chips

Group#1	Dose (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)	Time#2 (h)	Number of animals (Animal nos.) Male	Female	ID card color
E2	2	0.4	5	48	3 (00049–051)	3 (00106–108)	Blue

Housed by group in a polycarbonate cage with chips on a rubber mat heater (Mat no. 3)

Group#1	Dose (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)	Time#2 (h)	Number of animals (Animal nos.) #3 Male	Female	ID card color
F200	200	20	10	1,2,4,8,24	18 (00052–069)	18 (00109–126)	Orange

#1 Groups A0, C0: 0.5w/v% gum arabic solution by single oral administration;
Groups A100, A200, C100, C200, F200: OPC–14597 suspension by single oral administration;
Groups B200, D200: Reserpine suspension by single intraperitoneal injection;
Group E2: Mitomycin C solution by single intraperitoneal injection;

#2 Sacrifice time after administration at 10:59–12:29 on Day 1

#3 Three males and three females were sacrificed at each time for TK analysis by ascending of animal nos. The last three males and three females were dosed, but not used for TK analysis.

Analysis:

No. of replicates: peripheral blood samples [2 slides/animal] were prepared from 6/sex/grp [Grps A, B, C, D] or 3/sex [Grp E] at 48 hrs postdosing. [The sponsor noted that peripheral blood samples may be used for evaluation of micronuclei at 48 hrs after a single dose.] At 48 hrs postdosing, bone marrow samples were prepared from 2 femurs/animal [Grps A, B, C, D (6/sex/grp); Grp E (3/sex)]. Blood and bone marrow cells were stained with acridine orange for analysis.

Counting method: reticulocyte cts were based on examination of 300 erythrocytes ("and more")/animal. The number of micronucleated reticulocytes per animal was based on examination of 2000 reticulocytes using an "in-house computer system". The micronuclei were sized [large: 1-3 µm in major axis; small: <1 µm in major axis].

For analysis of bone marrow, the number of PCEs and NCEs was determined based on examination of 1000 erythrocytes/animal. The number of micronucleated PCEs was based on analysis of 2000 PCEs/animal, using an "in-house computer system". The micronuclei were categorized as either large or small as previously noted.

Criteria for positive results: the sponsor provided a description of the criteria for "prevention of hypothermia-related micronuclei induction of PIC-14597 with heating: (a) statistically significant increase in micronucleus frequency in Grp A, but not Grp C, (b) the micronucleus frequency in Grps A100 or A200 [but not Grps C100 and C200] exceeds

the upper limit ["mean + 2 standard deviations of 4.3% for males, 4.8% for females] of the in-house historical control data [negative control].

Deviations from protocol: not considered to affect results. It was noted that one deviation was that animals were fasted for 5 hrs prior to dosing [the sponsor noted that animals should not be fasted for longer than 5 hrs ("substantially") prior to dosing due to "abnormal cell growth"]. One CM was excluded from the analysis due to injury [fighting] and reduced body wt prior to necropsy.

Results

Mortality: there were no drug-related deaths.

Clinical signs: clinical signs were evident at both doses of aripiprazole and with reserpine in animals maintained at rm temperature. Reduced locomotor activity, ptosis, and crouched/prone position, and cold-to-touch was observed with both drugs, and at both doses of aripiprazole. With low-dose aripiprazole, females tended to be more affected than males. Additional signs observed at the HD of aripiprazole included wetness [abdomen, perineum], tremor on contact, and convulsion on contact [1 F]. Wetness [abdomen, perineum] and tremor on contact were also observed in a few animals treated with reserpine.

There were fewer clinical signs [and affected animals] in animals treated with aripiprazole and maintained under heated conditions. With aripiprazole, no clinical signs were evident at the LD in males; in LDF, reduced spontaneous motor activity and ptosis were observed in 1-2 of 6. At the HD, decreased motor activity was observed in 2/6 M and 2/6 F. With reserpine, decreased spontaneous motor activity, ptosis, tremor upon contact, and stool around the anus were observed in animals maintained under heated conditions.

Body wt: on Day 3, mean body wt was significantly reduced at the HD of aripiprazole and with reserpine in animals maintained at rm temperature [aripiprazole: 11-12%; reserpine: 14%], and in animals maintained under heated conditions [aripiprazole: 16-13%; reserpine: 19%].

Body temperature: the rectal temperature data were summarized in the following sponsor's tables:

Table 3-1
Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes after Single Oral Administration to ICR Mice with or without Heating
Item : Physiological Examination Summary - Body Temperature
Sex : Male Unit : ℃ Statistical Control : A0

Individually housed in steel cages

Dose Stage No of animals	Time	A0 Mean±S.D n	A100 Mean±S.D. n	A200 Mean±S.D. n
pre		36.80±0.59 6	36.35±0.60 6	37.05±0.52 6
2hr		36.37±0.54##	32.60±1.46**	28.65±1.68**
			6	6
4hr		36.17±0.53##	31.77±3.01**	26.20±1.44**
		6	6	6
8hr		36.40±0.53##	32.15±3.55**	24.77±0.27**
		6	6	6
12hr		36.83±0.79##	32.13±3.57**	24.70±0.21**
		6	6	6
24hr		36.53±1.13##	33.83±0.96	27.63±3.67**
		6	6	6
48hr		36.62±0.70##	36.43±0.92	33.05±2.23**
		6	6	6

A0 : 0mg/kg A100 : 100mg/kg A200 : 200mg/kg
* : P<0.05 , ** : P<0.01 : Significant difference from the control
# : P<0.05 , ## : P<0.01 : Significant dose dependency

Table 3-2
Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes after Single Oral Administration to ICR Mice with or without Heating
Item : Physiological Examination Summary - Body Temperature
Sex : Female Unit : ℃ Statistical Control : A0

Individually housed in steel cages

Dose Stage No of animals	Time	A0 Mean±S. D. n	A100 Mean±S. D. n	A200 Mean±S. D. n
pre		37. 17±0. 53 6	37. 48±0. 69 6	37. 58±0. 40 6
2hr		36. 67±0. 22## 6	31. 23±1. 67** 6	27. 30±1. 44** 6
4hr		37. 00±0. 30## 6	28. 38±3. 04** 6	24. 73±0. 58** 6
8hr		37. 03±0. 73## 6	28. 48±4. 37** 6	24. 55±0. 31** 6
12hr		37. 30±0. 65## 6	28. 58±3. 53** 6	24. 55±0. 14** 6
24hr		37. 57±0. 60## 6	33. 68±4. 51 6	28. 70±2. 95** 6
48hr		37. 42±0. 44# 6	37. 32±1. 37 6	33. 03±3. 93* 6

A0 : 0mg/kg A100 : 100mg/kg A200 : 200mg/kg
* : P<0. 05 , ** : P<0. 01 : Significant difference from the control
# : P<0. 05 , ## : P<0. 01 : Significant dose dependency

Table 3-3
Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes after Single Oral Administration to ICR Mice with or without Heating
Item : Physiological Examination Summary - Body Temperature
Sex : Male Unit : ℃ Statistical Control : A0

Individually housed in steel cages

Dose Stage No of animals	Time	A0 Mean±S. D. n	B200 Mean±S. D. n
pre		36. 80±0. 59 6	36. 52±0. 77 6
2hr		36. 37±0. 54 6	36. 25±0. 73 6
4hr		36. 17±0. 63 6	34. 77±1. 05* 6
8hr		36. 40±0. 53 6	28. 70±2. 34** 6
12hr		36. 83±0. 79 6	25. 90±2. 00** 6
24hr		36. 53±1. 13 6	26. 00±2. 81** 6
48hr		36. 62±0. 70 6	26. 08±2. 14** 6

A0 : 0mg/kg B200 : RSP200mg/kg
* : P<0. 05 , ** : P<0. 01 : Significant difference from the control

Table 3-4
Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes after Single Oral Administration to ICR Mice with or without Heating
Item : Physiological Examination Summary - Body Temperature
Sex : Male Unit : ℃ Statistical Control : C0

Housed by group in a polycarbonate cage on a rubber mat heater

Dose Stage No of animals	Time	C0 Mean±S. D. n	C100 Mean±S. D. n	C200 Mean±S. D. n
pre		36. 26±0. 60 5	36. 28±0. 38 6	36. 52±0. 68 6
2hr		36. 88±0. 55## 5	35. 97±0. 44 6	35. 53±0. 46** 6
4hr		36. 64±0. 44# 5	35. 27±0. 40** 6	35. 43±0. 46** 6
8hr		37. 50±0. 64 5	35. 78±0. 43** 6	36. 15±1. 09* 6
12hr		37. 38±0. 46# 5	35. 20±1. 01** 6	35. 48±1. 33* 6
24hr		37. 12±0. 28## 5	36. 00±0. 67* 6	35. 18±0. 88** 6
48hr		36. 94±0. 46 5	36. 88±1. 02 6	37. 47±0. 44 6

C0 : 0mg/kg heated C100 : 100mg/kg heated C200 : 200mg/kg heated
* : P<0. 05 , ** : P<0. 01 : Significant difference from the control
# : P<0. 05 , ## : P<0. 01 : Significant dose dependency

Table 3-6
Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes
after Single Oral Administration to ICR Mice with or without Heating
Item : Physiological Examination Summary - Body Temperature Housed by group in a polycarbonate cage on a rubber mat heater
Sex : Female Unit : ℃ Statistical Control : C0

Dose Stage Time No of animals	C0 Mean±S. D. n	C100 Mean±S. D. n	C200 Mean±S. D. n
pre	37.43±0.62 6	37.47±0.18 6	37.52±0.64 6
2hr	37.43±0.24##	35.93±0.52**	35.47±0.97**
4hr	37.28±0.48##	35.47±0.53**	34.78±1.19**
8hr	37.75±0.27##	36.13±0.45**	34.63±0.91**
12hr	37.77±0.37##	36.50±0.58*	35.33±1.01**
24hr	37.52±0.40##	37.07±0.39	36.55±0.45**
48hr	37.70±0.35	37.70±0.49	38.15±0.54

C0 : 0mg/kg heated C100 : 100mg/kg heated C200 : 200mg/kg heated
* : P<0.05 , ** : P<0.01 : Significant difference from the control
# : P<0.05 , ## : P<0.01 : Significant dose dependency

Table 3-6
Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes
after Single Oral Administration to ICR Mice with or without Heating
Item : Physiological Examination Summary - Body Temperature Housed by group in a polycarbonate cage on a rubber mat heater
Sex : Male Unit : ℃ Statistical Control : C0

Dose Stage Time No of animals	C0 Mean±S. D. n	D200 Mean±S. D. n
pre	36.26±0.60 5	36.68±0.39 6
2hr	36.68±0.55 5	36.88±0.63 6
4hr	36.64±0.44 5	36.73±0.53 6
8hr	37.50±0.64 5	37.20±0.60 6
12hr	37.38±0.46 5	36.92±1.06 6
24hr	37.12±0.28 5	35.92±1.11*
48hr	36.94±0.46 5	35.83±0.69*

C0 : 0mg/kg heated D200 : RSP200mg/kg heated
* : P<0.05 , ** : P<0.01 : Significant difference from the control

Micronuclei: cytotoxicity and micronucleated PCE [MN-PCE] data were summarized in the following sponsor's tables:

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Table 4-1 Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes after Single Oral Administration to ICR Mice with or without Heating

Items: Immature Erythrocyte Ratio in Erythrocytes and Micronucleus Frequency in Immature Erythrocytes
Sex: Male
Housing condition: Individually housed in steel cages

			A0	A100	A200	B200	E2
Peripheral blood	Immature Erythrocyte Ratio (%)	Mean	3.6	3.4	2.9	3.1	0.9*
		S.D.	1.6	0.7	0.4	0.3	0.3
		min-max	—	—			
		n	6	6	6	6	3
	Micronucleus Frequency (‰)	Total	20/12000	13/12000	24/12000	17/12000	207/6000**
		Mean	1.7	1.1	2.0	1.4	34.5
		S.D.	0.6	0.6	0.9	0.9	4.3
		min-max	—	—			
		n	6	6	6	6	3
Bone marrow	Immature Erythrocyte Ratio (%)	Mean	71	69	65	65*	37**
		S.D.	4	4	10	4	10
		min-max		—			
		n	6	6	6	6	3
	Micronucleus Frequency (‰)	Total	25/12000##	52/12000**	69/12000**	61/12000**	434/6000**
		Mean	2.1	4.3	5.8	5.1	72.3
		S.D.	0.7	2.0	3.3	2.1	22.9
		min-max		—			
		n	6	6	6	6	3

A0: 5% gum arabic solution, A100: OPC-14597-100mg/kg, A200: OPC-14597-200mg/kg
B200: Reserpine-200mg/kg, E2: Mitomycin C-2mg/kg

Micronucleated cells were counted in 2000 immature erythrocytes per mouse.
*: p<0.05, **:p<0.01: Significant difference from vehicle control (one tailed)
#: p<0.05, ##:p<0.01: Significant dose dependency (one tailed)

Table 4-2 Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes after Single Oral Administration to ICR Mice with or without Heating

Items: Immature Erythrocyte Ratio in Erythrocytes and Micronucleus Frequency in Immature Erythrocytes
Sex: Female
Housing condition: Individually housed in steel cages

			A0	A100	A200	E2
Peripheral blood	Immature Erythrocyte Ratio (%)	Mean	2.9	3.0	3.0	1.3**
		S.D.	0.5	0.2	0.3	0.5
		min-max	—			
		n	6	6	6	3
	Micronucleus Frequency (‰)	Total	21/12000	26/12000	22/12000	302/6000**
		Mean	1.8	2.2	1.8	50.3
		S.D.	0.5	0.8	0.7	16.4
		min-max	—			
		n	6	6	6	3
Bone marrow	Immature Erythrocyte Ratio (%)	Mean	70	64	68	53**
		S.D.	6	6	3	2
		min-max	—			
		n	6	6	6	3
	Micronucleus Frequency (‰)	Total	36/12000##	87/12000**	87/12000**	265/6000**
		Mean	3.0	7.3	7.3	44.2
		S.D.	0.9	3.4	3.1	8.8
		min-max	—			
		n	6	6	6	3

A0: 5% gum arabic solution, A100: OPC-14597-100mg/kg, A200: OPC-14597-200mg/kg
E2: Mitomycin C-2mg/kg

Micronucleated cells were counted in 2000 immature erythrocytes per mouse.
*: p<0.05, **:p<0.01: Significant difference from vehicle control (one tailed)
#: p<0.05, ##:p<0.01: Significant dose dependency (one tailed)

Table 4-3 Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes after Single Oral Administration to ICR Mice with or without Heating

Items: Immature Erythrocyte Ratio in Erythrocytes and Micronucleus Frequency in Immature Erythrocytes
Sex: Male
Housing condition: Housed by group in a cage on a rubber mat heater

			C0	C100	C200	D200
Peripheral blood	Immature Erythrocyte Ratio (%)	Mean	3.3	3.0	3.0	3.2
		S.D.	0.3	0.4	0.3	0.5
		min-max				
		n	5	6	6	6
	Micronucleus Frequency (‰)	Total	13/10000	12/12000	16/12000	23/12000
		Mean	1.3	1.0	1.3	1.9
		S.D.	0.4	0.3	0.7	1.0
		min-max				
		n	5	6	6	6
Bone marrow	Immature Erythrocyte Ratio (%)	Mean	74	62**	63**	60**
		S.D.	6	4	6	4
		min-max				
		n	5	6	6	6
	Micronucleus Frequency (‰)	Total	33/10000	38/12000	32/12000	32/12000
		Mean	3.3	3.2	2.7	2.7
		S.D.	1.7	1.3	1.1	0.4
		min-max				
		n	5	6	6	6

C0: 5% gum arabic solution, C100: OPC-14597-100mg/kg, C200: OPC-14597-200mg/kg
D200: Reserpine-200mg/kg

Micronucleated cells were counted in 2000 immature erythrocytes per mouse.
*: p<0.05, **:p<0.01: Significant difference from vehicle control (one tailed)
#: p<0.05, ##:p<0.01: Significant dose dependency (one tailed)

Table 4-4 Micronucleus Test of OPC-14597 in Peripheral Blood and Bone Marrow Erythrocytes after Single Oral Administration to ICR Mice with or without Heating

Items: Immature Erythrocyte Ratio in Erythrocytes and Micronucleus Frequency in Immature Erythrocytes
Sex: Female
Housing condition: Housed by group in a cage on a rubber mat heater

			C0	C100	C200
Peripheral blo:d	Immature Erythrocyte Ratio (%)	Mean	3.1##	2.9	2.7**
		S.D.	0.4	0.1	0.2
		min-max			
		n	6	6	6
	Micronucleus Frequency (‰)	Total	12/12000	14/12000	19/12000
		Mean	1.0	1.2	1.6
		S.D.	0.6	0.5	1.0
		min-max			
		n	6	6	6
Bone marrow	Immature Erythrocyte Ratio (%)	Mean	69	71	67
		S.D.	8	7	9
		min-max			
		n	6	6	6
	Micronucleus Frequency (‰)	Total	31/12000	35/12000	42/12000
		Mean	2.6	2.9	3.5
		S.D.	1.8	0.8	0.8
		min-max			
		n	6	6	6

C0: 5% gum arabic solution, C100: OPC-14597-100mg/kg, C200: OPC-14597-200mg/kg

Micronucleated cells were counted in 2000 immature erythrocytes per mouse.
*: p<0.05, **:p<0.01: Significant difference from vehicle control (one tailed)
#: p<0.05, ##:p<0.01: Significant dose dependency (one tailed)

TK: the data [from heated animals only] were summarized in the following table:

Table 5
Toxicokinetic Parameters of OPC-14597 in ICR Mice with Heating after Single Oral Administration at 200 mg/kg
Housing condition: Housed by group in a cage heated
Item: Plasma Concentration Parameter
Sex: Male

Compound	Cmax	Tmax	AUC(0-24hr)	AUC(0-48hr)
OPC-14597	5.8247	2	76.8576	96.0768
OPC-14857	0.8292	24	16.9959	28.0203
DM-1451	0.0087	2	0.1088	0.1520
DM-1452	0.7022	2	10.6874	13.5278
DCPP	0.2066	4	4.5310	7.2310
OPC-3373	1.2587	2	9.1099	10.5031

Sex: Female

Compound	Cmax	Tmax	AUC(0-24hr)	AUC(0-48hr)
OPC-14597	6.9756	4	67.8819	82.2627
OPC-14857	0.8726	4	11.6441	15.7349
DM-1451	0.0127	4	0.1768	0.2164
DM-1452	0.9435	4	11.6490	13.7874
DCPP	0.1699	4	2.4222	3.1014
OPC-3373	1.0865	4	11.2872	13.2288

4. **Study title: Chromosomal aberration test of 2,3-DCPP using cultured mammalian cells.**

Study no: 018329
Volume #: 103.3
Conducting laboratory and location: ——
Date of study initiation: 1/24/02
GLP: Y [OECD principles of GLP]
QA report: Y
Drug, lot #, radiolabel, and % purity: 2,3-DCPP, lot no. 0400000003, purity stated to be——

Formulation/vehicle: solution/distilled water.

Methods

Strains/species/cell line: Chinese hamster lung fibroblasts [CHL; doubling time: 15 hrs]
Dose selection criteria
Basis of dose selection: dose-range finding cytotoxicity assays: 20-160 µg/mL in the absence of S9 [7 concentrations], 20-320 µg/mL in the presence of S9 [9 concentrations]. Chromosomal aberrations were evaluated based on examination of 50 cells/concentration; MI was calculated based on examination of 500 cells/concentration.
Test agent stability: solution stated to be stable for 2 hrs.
Metabolic activation system: phenobarbital/5,6-benzoflavone-induced rat liver S9 [strain not specified]
Controls
Vehicle: distilled water

Negative controls: vehicle
Positive controls: MMC, CPA
Study design: in the definitive study, short-term and long-term treatments were evaluated.

Exposure conditions:

Incubation and sampling times:

short-term: cells were exposed to drug for 6 hrs in the absence of S9 and for 3 hrs in the presence of S9.
long-term: cells were exposed to drug for 24 hrs
confirmatory test: short-term treatment only, +/-S9

Doses used in definitive study:

short-term: 29.2, 40.8, 57.1, and 80 µg/mL [-S9], 30, 60, 90, and 120 µg/mL [+S9].
long-term: 5.0-60 µg/mL [duplicate cultures]
confirmatory test: same as for 1st short-term test.

Analysis

Results

Preliminary assay: with 6-hr incubation, %RCG was 35.6 and 59.4% [- and +S9, respectively] at 83.8 µg/mL. At the next higher concentration [168 µg/mL], %RCG was 1.3 and 12.9 [- and +S9, respectively]. With continuous treatment [24-hr], %RCG was 77.3% at 20.9 µg/mL and 7.2% at the next higher concentration [41.9 µg/mL].

Short-term [6 hr + 18 hr recovery]: the data for the cytotoxicity and chromosomal aberration assays are summarized in the following sponsor's tables:

cytotoxicity assay:

Table 2. Results of the second growth inhibition test of 2,3-DCPP in the short-term treatment method

Short-term (6 h) treatment with 18 h recovery without S9 mix						Short-term (6 h) treatment with 18 h recovery with S9 mix					
Dose (µg/mL)	Cytotoxicity			Aberrant cells c) (%)		Dose (µg/mL)	Cytotoxicity			Aberrant cells c) (%)	
	Cell growth rate a) (%)	Relative mitotic index b) (%)	Apparent mitotic cells density	Structural aberrant ions	Numerical aberrant ions		Cell growth rate a) (%)	Relative mitotic index b) (%)	Apparent mitotic cells density	Structural aberrant ions	Numerical aberrant ions
0	100	100	abundant	4	0	0	100	100	abundant	2	2
20.0	88.5	86.4	abundant	n.o.	n.o.	20.0	77.7	94.9	abundant	n.o.	n.o.
30.0	87.5	95.5	abundant	2	0	30.0	65.1	101.7	abundant	n.o.	n.o.
40.0	76.5	131.8	abundant	12	0	40.0	64.7	103.4	abundant	2	0
60.0	52.7	65.9	abundant	10	10	60.0	59.0	86.4	abundant	4	0
80.0	15.8	52.3	a few	8	2	80.0	62.6	110.2	abundant	14	2
120	2.0	n.o.	none	n.o.	n.o.	120	31.7	54.2	a few	8	2
160	0.9	n.o.	none	n.o.	n.o.	160	11.8	25.4	rare	n.o.	n.o.
						240	1.1	n.o.	none	n.o.	n.o.
						320	0.1	n.o.	none	n.o.	n.o.

a) Mean of two dishes, b) Calculated from the number of mitotic cells per 500 observed cells,
c) per 50 observed cells, n.o.: not observed.
Note: Apparent cytotoxicity was observed in the preliminary experiments with the maximum dose of 2,680 µg/mL as equivalent to 10 mmol/L and lower doses.

chromosomal aberration assays:

Chromosomal aberration test of 2,3-DCPP

Table 3. Treatment - Recovery 6-18 (h): S9 mix (-)

Dose (µg/mL)	# of cells	Structural aberrations						Gaps	Cell growth	Numerical aberrations		
		ctb	cte	csb	cse	oth	Total			Poly	Endo	Total
0	100	0	0	0	0	0	0	0	100	1	0	1
	100	1	0	0	0	0	1	0		0	0	0
	200	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)		1 (0.5)	0 (0.0)	1 (0.5)
29.2	100	2	1	0	0	0	3	0	82.2	0	0	0
	100	1	0	0	0	0	1	1	81.8	1	0	1
	200	3 (1.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.0)	1 (0.5)	(82.0)	1 (0.5)	0 (0.0)	1 (0.5)
40.8	100	2	0	0	0	0	2	0	54.9	2	0	2
	100	2	0	0	0	0	2	2	55.8	1	0	1
	200	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.0)	2 (1.0)	(55.4)	3 (1.5)	0 (0.0)	3 (1.5)
57.1	100	1	0	0	0	0	1	1	46.9	10	0	10
	100	1	0	0	0	0	1	0	46.4	16	0	16
	200	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	1 (0.5)	(46.7)	26 (13.0)	0 (0.0)	26 (13.0)
80.0	100	1	0	0	0	0	1	0	30.3	8	0	8
	100	1	1	0	0	0	2	0	17.5	22	0	22
	200	2 (1.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	0 (0.0)	(23.9)	30 (15.0)	0 (0.0)	30 (15.0)
(MMC) 0.1	100	9	32	8	0	0	43	0		0	0	0
	100	25	36	0	0	0	50	3		0	0	0
	200	34 (17.0)	68 (34.0)	8 (4.0)	0 (0.0)	0 (0.0)	93 (46.5)	3 (1.5)		0 (0.0)	0 (0.0)	0 (0.0)

Structural aberrations: ctb :chromatid break, cte :chromatid exchange, csb :chromosome break, cse :chromosome exchange, oth: others

Numerical aberrations: Poly :polyploidy, Endo: endoreduplication.

The values of the first and second lines come from each culture and those of the third line mean total.

The parenthesized values are the mean percentage.

MMC: Mitomycin C

Chromosomal aberration test of 2,3-DCPP

Table 3. (continued) Treatment - Recovery 6-18 (h): S9 mix (+)

Dose (µg/mL)	# of cells	Structural aberrations						Gaps	Cell growth	Numerical aberrations		
		ctb	cte	csb	cse	oth	Total			Poly	Endo	Total
0	100	2	0	0	0	0	2	0	100	1	0	1
	100	6	0	0	0	0	6	0		1	0	1
	200	8 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	8 (4.0)	0 (0.0)		2 (1.0)	0 (0.0)	2 (1.0)
30.0	100	2	0	0	0	0	2	0	86.8	0	0	0
	100	2	0	0	0	0	2	1	84.7	0	0	0
	200	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.0)	1 (0.5)	(85.8)	0 (0.0)	0 (0.0)	0 (0.0)
60.0	100	3	0	0	0	0	3	0	73.4	0	0	0
	100	3	1	0	0	0	4	0	67.6	0	0	0
	200	6 (3.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	7 (3.5)	0 (0.0)	(70.5)	0 (0.0)	0 (0.0)	0 (0.0)
90.0	100	1	1	0	0	0	2	0	57.1	1	0	1
	100	0	1	0	0	0	1	1	53.4	1	0	1
	200	1 (0.5)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	1 (0.5)	(55.3)	2 (1.0)	0 (0.0)	2 (1.0)
120	100	5	2	0	0	0	5	2	44.9	1	2	3
	100	7	0	1	0	0	8	3	39.2	2	0	2
	200	12 (6.0)	2 (1.0)	1 (0.5)	0 (0.0)	0 (0.0)	13 (6.5)	5 (2.5)	(42.1)	3 (1.5)	2 (1.0)	5 (2.5)
(CPA) 6	100	9	21	1	1	0	29	5		0	0	0
	100	4	18	2	0	0	22	0		0	0	0
	200	13 (6.5)	39 (19.5)	3 (1.5)	1 (0.5)	0 (0.0)	51 (25.5)	5 (2.5)		0 (0.0)	0 (0.0)	0 (0.0)

The legends are the same as Table 2.

CPA: Cyclophosphamide

Long-term [24-hr, -S9]: the data were summarized in the following sponsor's tables:

cytotoxicity assay:

Table 4. Results of the second growth inhibition test of 2,3-DCPP in the continuous treatment method

Continuous treatment without S9 mix (24-0 h)					
Dose (μg/mL)	Cytotoxicity			Aberrant cells c) (%)	
	Cell growth rate a) (%)	Relative mitotic index b) (%)	Apparent mitotic cells density	Structural aberrant ions	Numerical aberrant ions
0	100	100	abundant	2	0
5.00	84.6	45.8	abundant	4	0
7.50	76.3	32.2	abundant	4	0
10.0	71.4	35.6	a few	10	0
15.0	65.3	22.0	a few	14	0
20.0	49.3	11.9	rare	n.o.	n.o.
30.0	13.8	n.o.	none	n.o.	n.o.
40.0	1.6	n.o.	none	n.o.	n.o.
60.0	0.7	n.o.	none	n.o.	n.o.

( ) Treatment-recovery time,
a) Mean of two dishes, b) Calculated from the number of mitotic cells per 500 observed cells,
c) per 50 observed cells, n.o.: not observed.
Note: Apparent cytotoxicity was observed in the preliminary experiments with the maximum dose of 2680 μg/mL as equivalent to 10 mmol/L and lower doses.

chromosomal aberration assay:

Table 5. Treatment - Recovery 24-0 (h): S9 mix (-)

Dose (μg/mL)	# of cells	Structural aberrations						Gaps	Cell growth	Numerical aberrations		
		ctb	cte	csb	cse	oth	Total			Poly	Endo	Total
0	100	0	0	0	0	0	0	0	100	0	0	0
	100	1	0	0	0	0	1	0		1	0	1
	200	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)		1 (0.5)	0 (0.0)	1 (0.5)
6.67	100	2	0	1	1	0	4	0	76.4	0	0	0
	100	4	0	1	0	0	5	0	89.6	0	0	0
	200	6 (3.0)	0 (0.0)	2 (1.0)	1 (0.5)	0 (0.0)	9 (4.5)	0 (0.0)	(83.0)	0 (0.0)	0 (0.0)	0 (0.0)
10.0	100	2	1	0	0	0	3	0	70.1	0	0	0
	100	2	0	1	0	0	3	1	74.8	0	0	0
	200	4 (2.0)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	6 (3.0)	1 (0.5)	(72.5)	0 (0.0)	0 (0.0)	0 (0.0)
15.0	100	1	0	0	0	0	1	1	64.5	0	0	0
	100	3	1	1	0	0	5	0	61.7	2	0	2
	200	4 (2.0)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	6 (3.0)	1 (0.5)	(63.1)	2 (1.0)	0 (0.0)	2 (1.0)
(MMC)	100	32	53	1	0	0	68	1		0	0	0
	100	17	46	0	0	0	54	1		0	0	0
0.05	200	49 (24.5)	99 (49.5)	1 (0.5)	0 (0.0)	0 (0.0)	122 (61.0)	2 (1.0)		0 (0.0)	0 (0.0)	0 (0.0)

The legends are the same as Table 2.

The MI data from the short-term and long-term assays are summarized in the following sponsor's table:

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Table 6. Mitotic index in the chromosomal aberration test of 2,3-DCPP

Treatment method	Treatment- recovery time (h)	S9 mix	Dose (µg/mL)	Relative mitotic index (%)
Short-term treatment	6–18	Without	0	100
			29.2	111.5
			40.8	102.5
			57.1	86.9
			80.0	60.7
	6–18	With	0	100
			30.0	78.0
			60.0	76.0
			90.0	89.3
			120	67.3
Continuous treatment	24–0	Without	0	100
			6.67	37.5
			10.0	19.1
			15.0	13.8

Relative mitotic index was represented for the negative control as 100% in 1000 observed cells per dose.

confirmatory [6-hr]:

cytotoxicity [MI]:

Table 8. Mitotic index in the confirmatory chromosomal aberration test of 2,3-DCPP

Treatment method	Treatment- recovery time (h)	S9 mix	Dose (µg/mL)	Relative mitotic index (%)
Short-term treatment	6–18	Without	0	100
			29.2	80.5
			40.8	88.6
			57.1	70.5
			80.0	28.9
	6–18	With	0	100
			30.0	107.2
			60.0	106.6
			90.0	71.1
			120	34.9

Relative mitotic index was represented for the negative control as 100% in 1000 observed cells per dose.

chromosomal aberration assay:

Confirmatory chromosomal aberration test of 2,3-DCPP

Table 7. Treatment - Recovery 6-18 (h): S-9 mix (–)

Dose (µg/mL)	# of cells	Structural aberrations						Gaps	Cell growth	Numerical aberrations		
		ctb	cte	csb	cse	oth	Total			Poly	Endo	Total
0	100	1	0	0	0	0	1	0	100	1	0	1
	100	2	2	0	0	0	4	0		0	0	0
	200	3 (1.5)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.5)	0 (0.0)		1 (0.5)	0 (0.0)	1 (0.5)
29.2	100	2	1	0	0	0	3	0	77.4	1	0	1
	100	1	0	0	1	0	2	1	80.5	0	0	0
	200	3 (1.5)	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)	5 (2.5)	1 (0.5)	(79.0)	1 (0.5)	0 (0.0)	1 (0.5)
40.8	100	2	1	0	0	0	3	1	68.3	1	0	1
	100	0	1	0	0	0	1	0	68.1	2	0	2
	200	2 (1.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.0)	1 (0.5)	(68.2)	3 (1.5)	0 (0.0)	3 (1.5)
57.1	100	1	0	0	0	0	1	0	38.0	17	0	17
	100	0	0	0	0	0	0	0	39.4	19	0	19
	200	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	(38.7)	36 (18.0)	0 (0.0)	36 (18.0)
80.0	100	4	3	0	1	0	7	0	14.2	1	0	1
	100	4	5	0	1	0	10	0	19.5	6	0	6
	200	8 (4.0)	8 (4.0)	0 (0.0)	2 (1.0)	0 (0.0)	17 (8.5)	0 (0.0)	(16.9)	7 (3.5)	0 (0.0)	7 (3.5)
(MMC)	100	12	28	1	2	0	37	1		2	0	2
	100	19	29	3	0	0	45	1		0	0	0
0.1	200	31 (15.5)	57 (28.5)	4 (2.0)	2 (1.0)	0 (0.0)	82 (41.0)	2 (1.0)		2 (1.0)	0 (0.0)	2 (1.0)

The legends are the same as Table 2.

Table 7. (continued) Treatment - Recovery 6-18 (h): S-9 mix (+)

Dose (µg/mL)	# of cells	– Structural aberrations ctb	cte	csb	cse	oth	Total	Gaps	Cell growth	Numerical aberrations Poly	Endo	Total
0	100	2	0	1	0	0	3	0	100	0	0	0
	100	1	1	0	0	0	2	0		0	0	0
	200	3 (1.5)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	5 (2.5)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)
30.0	100	2	0	0	0	0	2	0	72.2	0	0	0
	100	6	0	0	0	0	6	0	76.0	2	0	2
	200	8 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	8 (4.0)	0 (0.0)	(74.1)	2 (1.0)	0 (0.0)	2 (1.0)
60.0	100	2	1	0	0	0	3	2	68.4	1	0	1
	100	4	0	0	0	0	4	1	69.3	0	0	0
	200	6 (3.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	7 (3.5)	3 (1.5)	(68.9)	1 (0.5)	0 (0.0)	1 (0.5)
90.0	100	1	1	0	0	0	2	0	50.0	3	0	3
	100	2	1	0	0	0	3	0	52.8	4	0	4
	200	3 (1.5)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.5)	0 (0.0)	(51.4)	7 (3.5)	0 (0.0)	7 (3.5)
120	100	4	6	1	0	0	11	2	29.9	1	0	1
	100	6	6	0	1	0	12	2	35.5	2	0	2
	200	10 (5.0)	12 (6.0)	1 (0.5)	1 (0.5)	0 (0.0)	23 (11.5)	4 (2.0)	(32.7)	3 (1.5)	0 (0.0)	3 (1.5)
(CPA) 6	100	14	8	2	1	0	25	1		1	0	1
	100	14	6	2	1	0	23	3		0	0	0
	200	28 (14.0)	14 (7.0)	4 (2.0)	2 (1.0)	0 (0.0)	48 (24.0)	4 (2.0)		1 (0.5)	0 (0.0)	1 (0.5)

The legends are the same as Table 2.

The sponsor considered the positive findings [for structural aberrations] observed in the first cytotoxicity assay to "...have occurred by chance..." since "...induction was not observed at any dose levels associated with the relative growth rate of 50% or higher in the chromosomal aberration test or confirmatory test". In addition, in the cytotoxicity assay, observations were based on examination of 50 cells/concentration, whereas for the other ["negative"] assays, 200 cells were examined per concentration.

The sponsor concluded that "From the results of this study, 2,3-DCPP was judged positive for the potential to induce chromosomal aberrations under the conditions of this experiment. However, the effect was considered to be secondary to cytotoxicity rather than evidence of direct DNA reactivity as it was induced only at doses resulting in greater than 50% inhibition of relative cell growth rate."

Genetic toxicology summary and conclusions

The sponsor conducted the following additional studies [submitted to the I42,776]: Ames test on metabolites, OPC-3373 and OPC-14857, an *in vitro* chromosomal aberration assay on metabolite, 2,3-DCPP [aka DCPP], and an *in vivo* micronucleus assay in mice.

Metabolites, OPC-3373 and OPC-14857, were negative in the Ames assay [*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. coli* WP2uvrA]. OPC-3373 was tested up to the maximum recommended concentration [5000 µg/plate], whereas OPC-14857 was tested up to concentrations associated with moderate-to-marked cytotoxicity.

2,3-DCPP was tested in an *in vitro* chromosomal aberration assay in CHL cells. 2,3-DCPP [aka DCPP] and DCPP-associated metabolites have been detected in human [and rat] urine. A number of assays were conducted in this study. In addition to the "definitive" assays, structural and numerical aberrations were scored in the cytotoxicity assays. The results of this study are summarized in the following table:

ASSAY	TREAT TIME [hr]	±S9	CONC [µg/mL]	% structural aberrations#	%numerical aberrations	relative cell growth
cytotoxicity	6	-	0	4	0	100
			30	2	0	87.5
			40	12	0	76.5
			60	10	10	52.7
			80	8	2	15.8
		+	0	2	2	100
			40	2	0	64.7
			60	4	0	59.0
			80	14	2	62.6
			120	8	2	31.7
definitive	6	-	0	0.5	0.5	100
			29.2	2	0.5	82.0
			40.8	2	1.5	55.4
			57.1	1	13.0	46.7
			80	1.5	15.0	23.9
		+	0	4	1	100
			30	2	0	85.8
			60	3.5	0	70.5
			90	1.5	1	55.3
			120	6.5	2.5	42.1
cytotoxicity	24	-	0	2	0	100
			5	4	0	84.6
			7.5	4	0	76.3
			10	10	0	71.4
			15	14	0	65.3
definitive	24	-	0	0.5	0.5	100
			6.67	4.5	0	83.0
			10	3.0	0	72.5
			15	3.0	1.0	63.1
definitive-repeat	6	-	0	2.5	0.5	100
			29.2	2.5	0.5	79.0
			40.8	2	1.5	68.2
			57.1	0.5	18.0	38.7
			80	8.5	3.5	16.9
		+	0	2.5	0	100
			30	4.0	1.0	74.1
			60	3.5	0.5	68.9
			90	2.5	3.5	51.4
			120	11.5	1.5	32.7

#does not appear to include gaps

2,3-DCPP produced increases in structural and numerical aberrations in one or more assays. With a short incubation period [6-hr], increases in % of cells with structural aberrations were observed in the initial cytotoxicity assay at concentrations not associated with excessive cytotoxicity, both in the absence and presence of metabolic activation. In the original definitive [i.e., chromosomal aberration assay], increases in structural aberrations were not observed [at similar concentrations as used in the cytotoxicity assay]. Sufficient cytotoxicity was observed in the original definitive assay, that is the relative cell growth [RCG] was <50% at the high concentrations tested [±S9]. In the repeat short-incubation assay, increases in structural aberrations were observed at the HCs tested, both without and with S9. However, the increases were associated with RCG of ≈17 and 33% in the absence and presence, respectively, of S9; the 17% value is clearly excessively cytotoxic and the 33% is close to the ≤30% which has been suggested as defining excessive cytotoxicity [although the definition of "excessive cytotoxicity" is controversial]. [Interestingly, a positive response was not observed in, for example, the definitive 6-hr

incubation assay at a concentration associated with an RCG of 24%.] Using continuous incubation, increases in structural aberrations were observed in the cytotoxicity assay [at concentrations associated with RCG of 71.4-65.3%], but not in the definitive assay. However, in the definitive continuous assay, sufficient cytotoxicity was not achieved at the HC tested.

The sponsor acknowledged that 2,3-DCPP was positive in the *in vitro* chromosomal aberration assay; however, the sponsor considered the positive response secondary to excessive cytotoxicity [defined as a >50% decrease in relative cell growth] rather than reflecting a clastogenic effect. The sponsor noted that 2,3-DCPP was primarily positive in the cytotoxicity assays in which only 50 cells/concentration were examined for the presence of micronuclei; in contrast, in the "negative" assays, 200 cells/concentration were examined. It is difficult to diminish the importance of the positive responses based on the fact that the majority of the increases in structural aberrations were detected when fewer cells were examined. Rather, it is presumed that the greater number of cells examined the greater the chance of detecting an effect. Also, the effects were still evident when the data were converted from percent to total number of affected cells.

2,3-DCPP was positive in this assay, although a positive effect was not consistently observed in all assays. The sponsor noted that positive responses were observed at cytotoxic concentrations; however, positive responses were observed at concentrations not associated with excessive cytotoxicity [e.g., 6-hr incubation cytotoxicity assay (±S9), 24-hr incubation cytotoxicity assay]. [Selected data from this study were conveyed to James T. MacGregor, Ph.D., D.A.B.T., an expert in genotoxicity, in a telecon; Dr. MacGregor concurred that 2,3-DCPP was positive for structural aberrations in this assay.] The definitive continuous assay was not adequate in that sufficiently high concentrations were not tested.

According to the OECD guidelines [Guideline 473: *In vitro* mammalian chromosome aberration test], "...this guideline is not designed to measure numerical aberrations and is not routinely used for that purpose". [It is assumed that "this guideline" refers to the assay.] It is of note, however, that 2,3-DCPP increased the % of cells with numerical aberrations in the absence of S9 in some of the assays, including both definitive studies using the short treatment time [cf. summary table].

Regarding the *in vivo* micronucleus assay conducted on aripiprazole, in the original NDA review it was noted that concentration-related increases in %MN-PCEs were observed in both males and females in one of two definitive assays. The sponsor attributed the positive responses to hypothermia. The sponsor did demonstrate that aripiprazole induced prolonged hypothermia; however, no study was conducted demonstrating that prevention of hypothermia prevented increases in MN-PCEs in the *in vivo* micronucleus assay. The sponsor has since conducted such a study. In this study, aripiprazole was administered at doses of 100 and 200 mg/kg p.o. to animals maintained either at rm temperature or under heated conditions. For comparison, reserpine (a drug previously demonstrated to produce hypothermia and a positive response in the *in vivo* micronucleus assay) was administered to separate grps of animals [M only]. Mitomycin C was used as a positive control. Observations consisted of the following: (a) clinical signs, (b) rectal temperature [prior to dosing, at 2-46 hrs postdosing], (c) analysis of micronuclei in peripheral blood and bone marrow cells [only at 48 hrs postdosing], (d) TK analysis was conducted on blood samples collected from separate grps of animals [heated condition only]. Regarding the sampling time for the peripheral blood and bone marrow cells, the sponsor noted only that peripheral blood may be sampled at 48 hrs after a single dose. This is correct; however, the OECD guidelines state that for both types of cells, samples should be taken "at least twice" after a single dose. For bone marrow, samples should be collected "...starting not earlier than 24 hrs after treatment, but not extending beyond 48 hours after treatment with appropriate interval(s) between the samples". For peripheral blood, "Samples...are taken at least twice, starting not earlier than 36 hours after treatment, with appropriate intervals following the first sample, but not extending beyond 72 hours". This is consistent with CFSAN's Redbook 2000

recommendations for the *in vivo* micronucleus assay. [Dr. MacGregor addressed this issue in his memo (Appendix 1).]

In animals maintained at rm temperature, aripiprazole produced dose-related decreases in rectal temperature. At 100 mg/kg, the greatest effect was observed at 4 hrs postdosing in males and females, although rectal temperature was significant reduced up to 12 hrs postdosing. At 200 mg/kg, the greatest effect was observed at 8-12 hrs postdosing, and rectal temperature was significantly reduced throughout the measurement period [up to 48 hrs postdosing]. Reserpine significantly decreased rectal temperature to an extent fairly similar to that observed with aripiprazole at 200 mg/kg. Neither aripiprazole nor reserpine produced any evidence of cytotoxicity or increases in micronuclei in peripheral blood erythrocytes. In contrast, the PC induced significant cytotoxicity and a marked increase in micronuclei. In bone marrow, aripiprazole exhibited no evidence of cytotoxicity; however, reserpine was cytotoxic at the dose tested. Aripiprazole and reserpine induced significant increases in micronuclei in bone marrow. MN-PCE frequency was significantly increased at both doses of aripiprazole. In males the effect was dose-related [although not dose-proportional]; in females, the MN-PCE frequency was the same at both doses.

In animals maintained under heated conditions, aripiprazole produced significant decreases in rectal temperature at both doses. The effect was prolonged [up to 12-24 hrs postdosing], but the magnitude of the effect was greatly attenuated compared to the effect in animals maintained at rm temperature [34-35° C vs 24-32° C]. In animals treated with reserpine, rectal temperature was significantly decreased, but only to ≈36° C and only at 24-48 hrs postdosing. In peripheral blood erythrocytes, aripiprazole exhibited cytotoxicity only in females, and only at 200 mg/kg. No evidence of cytotoxicity was observed with reserpine [in males]. Neither aripiprazole nor reserpine produced significant increases in micronuclei. However, the MN-PCE frequency tended to be higher in HDF [19/12000 vs 12/12000 for CF]. In bone marrow, aripiprazole significantly reduced the PCE:NCE ratio at both doses in males, but exhibited no evidence of cytotoxicity in females. Reserpine was cytotoxic at the dose tested [in males]. No significant increases in MN-PCE frequency were observed with either aripiprazole or reserpine. However, as in peripheral blood, MN-PCE frequency tended to be higher in HDF compared to CF [42/12000 vs 31/12000]. It is of note that mean body temperature was slightly lower in HDF compared to HDM.

The data from this study indicate that preventing hypothermia in rats attenuates the formation of MN-PCEs in animals treated with aripiprazole [and reserpine]. Considering that in humans, a decrease in core temperature to <35° C would not be tolerated, a positive response in the *in vivo* micronucleus due to the degree of hypothermia achieved in rats does not, in all probability, represent a risk to humans.

[Dr. MacGregor reviewed the study report for the *in vivo* micronucleus assay; his memo is provided in Appendix 1 of this review.]

Labeling recommendations

The following revisions to the sponsor's proposed labeling [dated 9/18/02] are recommended:

Mutagenesis

The mutagenic potential of aripiprazole was tested in the in vitro bacterial reverse-mutation assay, the in vitro bacterial DNA repair assay, the in vitro forward gene mutation assay in mouse lymphoma cells, the in vitro chromosomal aberration assay in Chinese hamster lung (CHL) cells, the in vivo micronucleus assay in mice, and the unscheduled DNA synthesis assay in rats.

Aripiprazole and a metabolite (2,3-DCPP) were clastogenic in the in vitro chromosomal aberration

assays in CHL cells with and without metabolic activation. **2,3-DCPP produced increases in numerical aberrations in the *in vitro* assay in CHL cells in the absence of metabolic activation. A positive response was obtained** — in the in vivo micronucleus assay in mice; **however, the response was shown to be due to a mechanism not considered relevant to humans.**

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V. CARCINOGENICITY

The only issue is one of labeling. The sponsor had one comment on the Carcinogenesis section of labeling.

Revision No. 23

The sponsor noted that "Increases in prolactin were not detected in the initial 4-week study in rats because the sampling timepoint was suboptimal relative to feed intake....At all doses tested in the 13-week study in rats, there were minimal increases (1.5- to 5-fold relative to concurrent controls) in prolactin at one or more timepoints during weeks 4 and 13 in the 13-week study".

Reviewer comment: serum prolactin was not significantly affected in females in either the 4- or 13-wk dietary study in F344 rats. Therefore, it is recommended that the labeling remain as originally recommended.

The sponsor provided interspecies comparisons based on AUC for the findings described in labeling. The sponsor noted that the AUCs in animals and humans were based on a sampling interval of 0-24 hrs.

Labeling recommendations

The following revisions to the sponsor's proposed labeling [dated 9/18/02] are recommended:

Carcinogenesis

Lifetime carcinogenicity studies were conducted in ICR mice and in Sprague-Dawley (SD) and F344 rats. Aripiprazole was administered for 2 years in the diet at doses of 1, 3, 10, and 30 mg/kg/day to ICR mice and 1, 3, and 10 mg/kg/day to F344 rats (0.2 to 5 and 0.3 to 3 times the maximum recommended human dose [MRHD] based on ~~a~~ mg/m^2 , respectively). In addition, SD rats were dosed orally for 2 years at 10, 20, 40, and 60 mg/kg/day (3 to 19 times the MRHD based on mg/m^2). Aripiprazole did not induce tumors in male mice or rats. In female mice, the incidences of pituitary gland adenomas and mammary gland adenocarcinomas and adenoacanthomas were increased at dietary doses of 3 to 30 mg/kg/day (0.1 to 0.9 times human exposure at MRHD based on AUC and 0.5 to 5 times the MRHD based on mg/m^2). In female rats, the incidence of mammary gland fibroadenomas was increased at a dietary dose of 10 mg/kg/day (0.1 times human exposure at MRHD based on AUC and 3 times the MRHD based on mg/m^2); and the incidences of adrenocortical carcinomas and combined adrenocortical adenomas/carcinomas were increased at an oral dose of 60 mg/kg/day (14 times human exposure at MRHD based on AUC and 19 times the MRHD based on mg/m^2).

Proliferative changes in the pituitary and mammary gland of rodents have been observed following chronic administration of other antipsychotic agents and are considered prolactin-mediated. Serum prolactin was not measured in the aripiprazole carcinogenicity studies. However, increases in serum prolactin levels were observed in female mice in a 13-week dietary study at the doses associated with mammary gland and pituitary tumors. Serum prolactin was not increased in female rats in ~~a~~ 4- and 13-week dietary stud~~ies~~ at the dose associated with mammary gland tumors. The relevance for human risk of the findings of prolactin-mediated endocrine tumors in rodents is unknown.

VI. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

The only issue is one of labeling. The sponsor made 4 comments to this section of labeling.

1. Revision No. 25

The sponsor provided relevant historical control data for fused sternabrae in New Zealand White rabbits from studies conducted in 1988-1994 [84 litters, 667 fetuses] in the same laboratory in which the embryofetal development study in rabbits was conducted. The overall incidence of fused sternabrae was 0.75%. The highest fetal and litter incidences were 1.98 and 15.38%, respectively. The sponsor also provided historical control data in NZW rabbits collected by MARTA [Middle Atlantic Reproduction and Teratology Association] and MTA [Midwest Teratology Association]. According to the MARTA/MTA database [data were not provided for each source separately], the incidence of fused sternabrae was 0.674 ± 0.92% [mean ± SD] for fetuses and 4.635 ± 5.94% [mean ± SD] for litters. The maximum incidence was 5 and 23.5% for affected fetuses and litters, respectively.

The sponsor noted that "Both fetal and litter incidences of fused sternabrae at 30 mg/kg/day of aripiprazole (3.7 and 23.5%, respectively) in the Segment II study in rabbits are within the upper limit of the historical control range (as reported by MARTA/MTA) for this fetal skeletal alternation in NZW rabbits".

Reviewer comment: the fetal and litter incidences of fused sternabrae observed at the mid- [30 mg/kg] and high-dose [100 mg/kg] of aripiprazole exceed the concurrent control and the laboratory's own HC incidences; therefore, labeling should be revised to indicate the occurrence of fused sternabrae at both doses. The sponsor provided interspecies comparisons based on plasma AUC data [for parent compound]. The AUC data were obtained from a 13-day TK study conducted at doses of 10, 30, and 100 mg/kg p.o. [$AUC_{(0\text{-}24hr)}$ = 16442, 21150, and 85961 ng•hr/mL, respectively]. Plasma exposure at the LD was disproportionate compared to exposure at the other doses; dose-corrected exposure was 1644, 705, and 860 at 10, 30, and 100 mg/kg, respectively. Whether or not this was due to high variability at the lower exposure is not clear.

2. Revision No. 26

The sponsor noted that the "The definition for hepatodiaphragmatic nodules provided in parentheses is incorrect. Hepatodiaphragmatic nodules develop on the parietal surface of the liver proximal to the diaphragm but do not by definition protrude through it unless there is an existing diaphgragmatic [sic] hernia".

Reviewer comment: based on the information provided in the original NDA, the relationship between the findings of "hepatodiaphragmatic nodules" and "liver protrusion through the diaphragm" was unclear. The additional information provided supports the sponsor's labeling revision.

3. Revision No. 27

Regarding the results of the embryofetal development findings in rats, the sponsor noted that "...maternal toxicity was slight at 10 mg/kg/day", but that "it was more severe at 30 mg/kg/day..." based on "...slight body wt loss on days 8-11 gestation (early in dosing), body weight gain suppression of 36-80% during the dosing period, and decreased body weight (8%) and food consumption (13%) relative to controls at the end of the dosing period". Based on these effects, the sponsor proposed "...that the last sentence of the paragraph detailing the findings in the developmental toxicity study in rats be changed to reflect that

the decreased fetal weights, undescended testes, delayed ossification, and vaginal opening were at least, in part, a consequence of dose-related maternal toxicity".

Reviewer comments: the sponsor's characterization of maternal toxicity as "Dose-related" exceeds the effects observed. There were no significant drug-related effects characteristic of maternal toxicity at the 10-mg/kg dose. There was evidence of maternal toxicity at the 30-mg/kg dose, e.g., significant decrease in body wt gain. The sponsor's revision suggesting an association between maternal toxicity and some of the developmental effects observed in rats is not supported by the data.

4. Revision No. 28

The sponsor noted an omission of standard labeling [last paragraph under the Pregnancy subsection].

Reviewer comment: the sponsor is correct that this wording was mistakenly omitted.

Labeling recommendations

The following revisions to the sponsor's proposed labeling [dated 9/18/02] are recommended [Dr. Sonia Tabacova has reviewed the information submitted by the sponsor relevant to Reproductive and Developmental Toxicology, and concurs with the following recommendations]:

"Impairment of fertility

Female rats were treated with oral doses of 2, 6, and 20 mg/kg/day (0.6, 2, and 6 times the maximum recommended human dose [MRHD] on a mg/m^2 basis) of aripiprazole from 2 weeks prior to mating through day 7 of gestation. Estrus cycle irregularities and increased corpora lutea were seen at all doses, but no impairment of fertility was seen. Increased pre-implantation loss was seen at 6 and 20 mg/kg, and decreased fetal weight was seen at 20 mg/kg.

Male rats were treated with oral doses of 20, 40, and 60 mg/kg/day (6, 13, and 19 times the MRHD on a mg/m^2 basis) of aripiprazole from 9 weeks prior to mating through mating. Disturbances in spermatogenesis were seen at 60 mg/kg, and prostate atrophy was seen at 40 and 60 mg/kg, but no impairment of fertility was seen.

Pregnancy

Pregnancy Category C

In animal studies aripiprazole demonstrated developmental toxicity, including possible teratogenic effects in rats
and bbits

Pregnant rats were treated with oral doses of 3, 10, and 30 mg/kg/day (1, 3, and 10 times the **maximum recommended human dose** [MRHD] on a mg/m^2 basis) of aripiprazole during the period of organogenesis. Gestation was slightly prolonged at 30 mg/kg. Treatment caused a slight delay in fetal development as evidenced by decreased fetal weight (30 mg/kg), undescended testes (30 mg/kg), and

delayed skeletal ossification (10 and 30 mg/kg). There were no adverse effects on embryofetal or pup survival. Delivered offspring had decreased bodyweights (10 and 30 mg/kg), and increased incidences of hepatodiaphragmatic nodules and diaphragmatic hernia at 30 mg/kg; (the other dose groups were not examined for these findings). (A low incidence of diaphragmatic hernia was also seen in the fetuses exposed to 30 mg/kg.) Postnatally, delayed vaginal opening was seen at 10 and 30 mg/kg and impaired reproductive performance (decreased fertility rate, corpora lutea, implants, and live fetuses, and increased post-implantation loss likely mediated through effects on female offspring) was seen at 30 mg/kg.

Some maternal toxicity was seen at 30 mg/kg; however, there was no evidence to suggest that the developmental effects were secondary to maternal toxicity.

Pregnant rabbits were treated with oral doses of 10, 30, and 100 mg/kg/day (2, 3, and 11 times human exposure at MRHD based on AUC and 6, 19, and 65 times the MRHD based on mg/m^2) of aripiprazole during the period of organogenesis. Decreased maternal food consumption and increased abortions were seen at 100 mg/kg. Treatment caused increased fetal mortality (100 mg/kg), decreased fetal weight (30 and 100 mg/kg), increased incidence of a skeletal abnormality (fused sternebrae **at 30 and 100 mg/kg**), and minor skeletal variations (100 mg/kg).

In a study in which rats were treated with oral doses of 3, 10, and 30 mg/kg/day (1, 3, and 10 times the MRHD on a mg/m^2 basis) of aripiprazole peri- and post-natally (from day 17 of gestation through day 21 post-partum), slight maternal toxicity and slightly prolonged gestation were seen at 30 mg/kg. An increase in stillbirths, and decreases in pup weight (persisting into adulthood) and survival, were seen at this dose.

There are no adequate and well-controlled studies in pregnant women. It is not known whether aripiprazole can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. Aripiprazole should be used during pregnancy only if the potential benefit outweighs the potential risk to the fetus."

APPEARS THIS WAY
ON ORIGINAL

VII. SPECIAL TOXICOLOGY

1. Study title: BMS-337039: neutral red uptake phototoxicity assay in BALB/c 3T3 mouse fibroblasts.

Study no: 02AC91.140056 [DM02028]
Volume #: 103.1
Conducting laboratory and location: [redacted]
Date of study initiation: 5/10/02
GLP: Y
QA report: Y
Drug, lot #, radiolabel, and % purity: BMS-337039 [OPC-31, OPC-14597, aripiprazole]
Formulation/vehicle: solution/DMSO

Methods

[redacted]

Three definitive assays were performed. Aripiprazole was tested at concentrations of 1.77-56.2 µg/mL, based on the results of the range-finding study. The first 2 assays were conducted simultaneously, but were independent. The 3rd assay was conducted in order to confirm the results of the 1st assay, since the PC data were somewhat lower than the HC range in the 1st assay. However, similar PC data were obtained in the 3rd assay. The sponsor considered all three assays definitive since the HC "data set is limited and under development..."

Results

Range-finding study: aripiprazole was cytotoxic at concentrations of 30 and 100 µg/mL; however, there was no evidence of phototoxicity. Chlorpromazine was cytotoxic at concentrations of 56.2 and 100 µg/mL in the absence of UVA. In the presence of UVA, cytotoxicity was observed with chlorpromazine at concentrations of 3.14-9.96 µg/mL, indicating a phototoxic effect.

Definitive studies: no evidence of phototoxicity was observed with aripiprazole in the 3 definitive studies. In contrast, chlorpromazine met the criteria for phototoxicity [based on PIF and MPE] in all three assays. The data were summarized in the following sponsor's tables:

Sponsor's Designation	IC_{50} (µg/ml)			pH in HBSS (highest tested dose)
BMS-337039 (aripiprazole, OPC-31, OPC-14597)	Range-Finding	Phototox (+UVA)	15.442	6.5
		Cytotox (-UVA)	14.453	
	Trial 1 (B1)	Phototox (+UVA)	15.969	6.5
		Cytotox (-UVA)	16.072	
	Trial 2 (B2)	Phototox (+UVA)	15.180	6.5
		Cytotox (-UVA)	14.741	
	Trial 3 (B3)	Phototox (+UVA)	15.697	6.5
		Cytotox (-UVA)	21.485	
Chlorpromazine	Range-Finding	Phototox (+UVA)	2.3050	NA
		Cytotox (-UVA)	38.996	
	Trial 1 (B1)	Phototox (+UVA)	2.4996	
		Cytotox (-UVA)	31.104	
	Trial 2 (B2)	Phototox (+UVA)	2.2842	
		Cytotox (-UVA)	32.294	
	Trial 3 (B3)	Phototox (+UVA)	1.3112	
		Cytotox (-UVA)	12.021	

NA- Not Applicable

MEAN PHOTO EFFECT AND PHOTO-IRRITANCY FACTOR SUMMARY

Sponsor's Designation	MPE [1]				PIF [2]			
	Range-Finding	Trial 1	Trial 2	Trial 3	Range-Finding	Trial 1	Trial 2	Trial 3
BMS-337039 (aripiprazole, OPC-31, OPC-14597)	-0.006	-0.028	0.007	0.046	0.942	1.007	0.972	1.370
Chlorpromazine	0.488	0.306	0.497	0.325	16.919	12.447	14.142	9.168

1 - Mean Photo Effect: a material is considered nonphototoxic if the MPE is < 0.1 and phototoxic if the MPE is ≥ 0.1.
2 - Photo-Irritancy Factor: a material is considered phototoxic if the PIF > 5.0.

2. Study title: Four-week oral ultrastructural investigative study of adrenocortical hypertrophy in female rats.

Study no: DM02021
Volume #: 103.2
Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute, IN
Date of study initiation:
GLP: Y
QA report: Y
Drug, lot #, radiolabel, and % purity: BMS-337039 [OPC-31, OPC-14597, aripiprazole]
Formulation/vehicle: suspension/5% gum arabic

Methods

Results: there were no unscheduled deaths. At necropsy, bilateral diffuse [minimal] dark discoloration of the adrenal glands was noted in 5/15 DTF. Upon microscopic examination, adrenal gland hypertrophy was noted in 11/15 DTF; the finding was characterized as minimal in 5/15 DTF and as mild in 6/15 DTF. No findings were evident in 5/5 CF. As determined by EM analysis, the hypertrophy of the zona fasciculata and the zona reticularis was "...mainly due to increases (number and size) in the mitochondrial compartment and proliferation of the SER. Other notable changes included reductions in cyotoplasmic lipid droplets and hypertrophy of the juxtanuclear Golgi complex..."

The sponsor concluded that the adrenal hypertrophy observed was consistent with prolonged ACTH stimulation, the decrease in cellular lipids reflecting increased synthesis of steroid hormones and cell membrane. It was noted that "...BMX-337039 has been shown to increase plasma ACTH and serum corticosterone in female rats given 60 mg/kg/day for 4 and 13 weeks and in male rats administered 40 and 60 mg/kg/day for 13 weeks" [cf Study DM01013]. This effect was considered to be mediated via agonist effects at the $5HT_{1A}$ receptor. It was noted that a similar finding was observed in rats administered ziprasidone [which has "potent" $5HT_{1A}$ agonist activity] at doses of 40 and 200 mg/kg/day. However, it was also noted that "...the mechanism for the adrenocortical hypertrophic response in rats given BMS-337039 may be multifactorial since a contributory effect of prolonged ACTH stimulation due to stress at overtly toxic doses cannot be ruled out".

Special toxicology summary and conclusions

Apparently, phototoxicity testing was conducted on aripiprazole in response to a request by EMFA. According to the Guidance on Photosafety Testing [Committee for Proprietary Medicinal Products, The European Agency for the Evaluation of Medicinal Products], if a need for phototoxicity testing is warranted, "...a basic phototsafety testing should include studies for the evaluation of the phototoxic, photogenotoxic and photoallergy potential. Positive findings in these studies should trigger the incorporation of phototoxicity endpoints into safety monitoring in clinical trials". Only phototoxicity was assessed for aripiprazole. No evidence of phototoxicity was observed with aripiprazole in 3 definitive studies.

The sponsor also conducted a 4-wk oral study in female Sprague-Dawley rats to further investigate the adrenal effects observed with aripiprazole in other toxicity studies and in a rat carcinogenicity study. Aripiprazole was administered by gavage at a dose of 60 mg/kg [the HD in the carcinogenicity study in Sprague-Dawley rat]. Observations consisted of clinical signs, body wt, gross pathology, histopathology, and EM. [Terminal studies were conducted only on adrenal gland.] Using light microscopy, adrenal gland hypertrophy of the zona fasciculata and zona reticularis was observed in a majority of drug-treated F. Upon further examination using EM, the hypertrophy was determined to be due primarily to increases in "the mitochondrial compartment and proliferation of the SER". It was the sponsor's opinion that these changes were consistent with prolonged ACTH stimulation, an effect attributed to $5HT_{1A}$ agonist effects of aripiprazole. The sponsor did not quantitate ACTH in this study. As this reviewer noted in the original review, "...the relationship between elevated ACTH levels and adrenocortical tumors is unclear". The data from the 4-wk special study do not provide sufficient data to conclude that elevations in ACTH underlie the adrenal gland effects observed [in particular, the adrenocortical tumors in female Sprague-Dawley rats].

VIII. SUMMARY OF LABELING RECOMMENDATIONS

The following revisions to the sponsor's proposed labeling [version dated 9/18/02] are recommended:

CLINICAL PHARMACOLOGY

Pharmacodynamics

Aripiprazole exhibits high affinity for dopamine D_2 and D_3, serotonin 5-HT_{1A} and 5-HT_{2A} receptors (K_i values of 0.34, 0.8, 1.7, and 3.4 nM, respectively), moderate affinity for dopamine D_4, serotonin 5-HT_{2C} and 5-HT_7, alpha$_1$-adrenergic and histamine H_1 receptors (K_i values of 44, 15, 39, 57, and 61 nM, respectively), and moderate affinity for the serotonin reuptake site (K_i = 98 nM). Aripiprazole has no appreciable affinity for cholinergic muscarinic receptors (IC_{50} > 1000 nM). Aripiprazole functions as a partial agonist at the dopamine D_2 and the serotonin 5-HT_{1A} receptors, and as an antagonist at serotonin 5-HT_{2A} receptor.

The mechanism of action of aripiprazole, as with other drugs having efficacy in schizophrenia, is unknown.

Aripiprazole's antagonism of adrenergic receptors may explain the orthostatic hypotension observed with this drug.

[There is no objection to removing the discussion of retinal pathology in albino rats from the PRECAUTIONS section.]

Carcinogenesis

Lifetime carcinogenicity studies were conducted in ICR mice and in Sprague-Dawley (SD) and F344 rats. Aripiprazole was administered for 2 years in the diet at doses of 1, 3, 10, and 30 mg/kg/day to ICR mice and 1, 3, and 10 mg/kg/day to F344 rats (0.2 to 5 and 0.3 to 3 times the maximum recommended human dose [MRHD] based on ~~a~~ mg/m^2, respectively). In addition, SD rats were dosed orally for 2 years at 10, 20, 40, and 60 mg/kg/day (3 to 19 times the MRHD based on mg/m^2). Aripiprazole did not induce tumors in male mice or rats. In female mice, the incidences of pituitary gland adenomas and mammary gland adenocarcinomas and adenoacanthomas were increased at dietary doses of 3 to 30 mg/kg/day (0.1 to 0.9 times human exposure at MRHD based on AUC and 0.5 to 5 times the MRHD based on mg/m^2). In female rats, the incidence of mammary gland fibroadenomas was increased at a dietary dose of 10 mg/kg/day (0.1 times human exposure at MRHD based on AUC and 3 times the MRHD based on mg/m^2); and the incidences of adrenocortical carcinomas and combined adrenocortical adenomas/carcinomas were increased at an oral dose of 60 mg/kg/day (14 times human exposure at MRHD based on AUC and 19 times the MRHD based on mg/m^2).

Proliferative changes in the pituitary and mammary gland of rodents have been observed following chronic administration of other antipsychotic agents and are considered prolactin-mediated.