

loss of the germinal epithelium in affected seminiferous tubules. "The epididymal ducts of [the affected] animals and seven additional high-dose males contained minimally to moderately increased numbers of exfoliated spermatogenic epithelial cells."(d) subchronic inflammation of the prostate (ampullary gland) "...involved a small area of one of the glands and was characterized by the infiltration of macrophages and other inflammatory cells in acini that contained displaced spermatozoa."

The pituitary atrophy was attributed to D₂ agonist properties [i.e., inhibition of prolactin release] of aripiprazole. However, the effects observed on the male reproductive organs and mammary gland were considered due to "hormonal imbalance". Although the findings primarily appeared to reflect hyperprolactinemia, the sponsor did note that "microscopic testicular changes similar to those of the present study were observed in male swine after prolactin-induced hyperprolactinemia and bromocriptine-induced hypoprolactinemia". The sponsor noted that mammary gland atrophy was "also" noted in male rats treated for 6 months with quetiapine. With aripiprazole, mammary gland atrophy was noted only at doses of 10 and 60 mg/kg (0/20 CM, 4/20 LDM, 0/20 MDM, and 4/20 HDM).] The inflammatory effect in prostate was not considered to reflect a hormonal effect. The sponsor noted that a similar effect was observed with risperidone.

The effects on female reproductive organs and mammary gland were considered consistent with hyperprolactinemia. Adrenal hypertrophy were considered to be indirectly related to drug-induced pharmacologic effects [e.g., 5HT_{1A} agonist activity (which has been shown to produce increases in plasma ACTH and corticosterone levels in rats)]. The sponsor noted that similar findings were observed with quetiapine [after 6 months of treatment]. Lipofuscin deposition was considered to reflect increased turnover of cellular organelles.

EM: sections of lung from 4 HD animals [#1404, 1411, 2415, 2420] were examined by EM.

Upon ultrastructural examination, the following was noted: "...focal accumulation of alveolar histiocytes (macrophages) often in expanded alveolar spaces. These histiocytes contained a single small round to oval nucleus with marginated chromatin and abundant cytoplasm with multiple vacuolated spaces, which presumably were due to loss of lipid material during fixation and processing...fragments of lamellar electron-dense material, interpreted as myelin membranes, were noted in some of the cytoplasmic vacuolar spaces." It was noted that these findings were consistent with those "...associated with spontaneous pulmonary histiocytosis in rats..."

Immunohistochemistry: the data were summarized in the following sponsor's table:

Table 1: Group mean of Ki-67 immunopositive adrenocortical cells per standard image field in the outer zona fasciculata and zona reticularis

	Control		BMS-337039 (60 mg/kg/day)	
	male	female	male	female
Outer zona fasciculata	2.8 ± 1.2 [9]	11.5 ± 4.7 [10]	2.7 ± 2.9 [9] <i>p</i> = 0.96	4.5 ± 2.3 [9] <i>p</i> = 0.0009
Zona reticularis	0.8 ± 0.5 [9]	1.6 ± 1.5 [9]	0.2 ± 0.2 [8] <i>p</i> = 0.009	1.5 ± 1.5 [8] <i>p</i> = 0.94

Data expressed as mean ± standard deviation; [number of animals]; Student t-test comparison to same sex control; Student t-test analysis incorporated results of F-test to compare variances (not shown).

Adrenocortical cell proliferation was significantly decreased in the outer zona fasciculata in HDF and in the zona reticularis in HDM. The sponsor noted that the decrease in cell proliferation in females may have been due to the fact that adrenal glands in HDF were hypertrophic and that, therefore, a few number of cells per area were counted per unit area. The effect in males was considered spurious since the outer zona fasciculata, "the major zone of cell proliferation...", was not affected

Toxicokinetics: the data were provided in a separate report.

Monkey

1. a 52-wk oral (gavage) toxicity study was conducted in cynomolgus monkey [4/sex/grp] at doses of 0, 0.5, 5, and 25 mg/kg. [OPC-31 lot 1E79M was used]. Observations consisted of the following: clinical signs, body wt, food consumption, ophthalmology, ECG, TK [samples collected at 2, 4, 8, and 24 hrs postdosing on Day 1 and during Wks 24 and 50], hematology [hgb, hct, rbc ct, MCH, MCHC, MCV, thrombocyte ct, wbc ct (total, differential), PT, APTT, reticulocyte ct], clinical chemistry [BUN, ALT, "AFOS", AST, albumin, CPK, LDH, Ca, Na, K, Cl, ChE, bilirubin, globulin, glucose, creatinine, PL, cholesterol, P_i, total protein, TG, leucine aminopeptidase, GGT], urinalysis [pH, protein, glucose, bilirubin, volume, blood (Hgb), crystals, specific gravity, wbcs, ketone bodies, urobilinogen, rbcs, epithelial cells], terminal studies [organ wt, gross and histopathology (adrenals, aorta bone, bone marrow, brain, bronchi, cecum, colon, duodenum, epididymides, esophagus, eyes, gallbladder, heart, ileum, jejunum, kidney, lacrimal glands, liver, lung, lymph node, mammary glands, ovary, pancreas, parathyroids, pituitary, prostate, rectum, salivary gland, seminal vesicle, spinal cord, skeletal muscle, skin, spleen, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina)].

There were no unscheduled deaths. Drug-related clinical signs were evident at all but the LD. Clinical signs observed at both the MD and HD consisted of tremors, catalepsy, impaired motor activity, hyporeactivity, and abnormal posture. Transient body wt loss was observed in HD animals; however, at the end of the dosing period, body wts were similar among grps. A transient decrease (during the 1st wk only) in food consumption was observed in a few HD animals. No drug-related effects were observed on ophthalmologic, ECG, hematology, or urinalysis parameters. There were also no clear drug-related effects on clinical chemistry parameters. There were no drug-related effects on absolute organ wts; however, relative wts of seminal vesicles, prostate, and/or testis were reduced in MDM and HDM and relative ovary and thyroid wts were reduced in HDF. There were no clear drug-related microscopic findings. Microscopic findings consisted of changes in one or more male reproductive organs [e.g., "...absence of spermatozoa in some seminiferous tubules...", shortening and flattening of the glandular epithelium of the seminal vesicle, or immaturity of the prostate and seminal vesicles] in selected animals; however, the effects were either not dose-related or were observed only sporadically.

Plasma levels of OPC-31 were <LLOD at the LD except during Wk 50 in 1 animal. C_{max} was 81-89 ng/mL in MDM [no accumulation was noted during the dosing period] and 85, 148 and 191 ng/mL in MDF on Day 1, Wk 24, and Wk 50, respectively. C_{max} increased with the duration of dosing at the HD. "The overall range in the 2- and 4-hour samples in Week 50 was 337-1293 ng/ml for all HD males and was 88-1103 for all HD females".

2. Study title: **39-week oral gavage toxicity study in cynomolgus monkey** [Study no: 99354 Study No. 6108-335, Volume #1.57-1.61, Conducting laboratory and location: _____, Date of study initiation: 12/13/99, **GLP except for analysis of bile, gallstone and gallsand and TK analyses, QA report:Y**]

Drug, lot #, and % purity: BMS-337039, lot no. C99G74M, _____
Formulation/vehicle: suspension/5% gum arabic

Methods**Dosing:**

Species/strain: cynomolgus monkey _____]
#/sex/group or time point (main study): 4/sex/grp
Satellite groups used for toxicokinetics or recovery: no
Age: 2-4 yrs
Weight: 1.9-3.2 kg
Doses in administered units: 0, 25, 50, 100/75 mg/kg. HD animals received one 100-mg/kg dose [Day 1], and received no doses on Days 2-4. The HD was lowered to 75 mg/kg on Day 5.
Route, form, volume, and infusion rate: oral/gavage/5 mL per kg. It was noted that "...nasal gavage was used when animals struggled or vomited excessively during dosing..." The dosing suspension was stirred during drug administration periods. Samples were collected for analysis of homogeneity and concentration during Wks 1, 6, 13, and 39, and for assessment of stability during Wk 1 [15-day storage]. These analyses were conducted by the sponsor, _____ Actual drug concentrations were reported to be "...consistently 95% to 105% of target..."

Observations and times

Clinical signs: animals were observed twice daily for mortality and morbidity and once daily for clinical signs. Physical examinations [including rectal temperature, respiration rates] were performed prior to start of dosing and during Wks 13, 26, and 39.

Body weights: body wts were recorded prior to the start of dosing, and weekly from Day 1 of dosing on.

Food consumption: food intake was assessed qualitatively once a day.

Ophthalmoscopy: examinations were performed on all animals prior to the start of dosing and during Wks 13, 26, and 39. Mydriasis was induced for examination of "...the anterior portion of the eye, optic media, and ocular fundus..." by indirect ophthalmoscope.

ECG: ECG, bp, and hr were recorded in all animals prior to the start of dosing and during Wks 13, 26, and 39. Animals were examined while awake using ten leads [nos]. The timing of recordings in relation to daily dose was not specified except that they were conducted after dosing.

Clinical pathology: blood and urine samples were collected from all animals once prior to the start of dosing, during Wks 13 and 26, and on day of scheduled sacrifice for analysis of the following parameters:

Hematology: rbc ct, hgb, hct, MCV, MCHC, MCH, platelet ct, wbc ct [total, differential], blood cell morphology, PT, APTT, fibrinogen.

Clinical chemistry: glucose, urea N, creatinine, total protein, albumin, globulin, A/G ratio, total bilirubin, cholesterol, TG, AST, ALT, alkaline phosphatase, GGT, Ca, P_i, Na, K, Cl.

Urinalysis: volume [16-hr collection], specific gravity, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, microscopic analysis of sediment.

Gross pathology: a complete necropsy was performed on all animals, including those that died or were sacrificed prematurely. One HDF [#I06740] was sacrificed during Wk 3 and was replaced with F#I06742. Bile ["as much...as possible..."] was collected from all animals and stored for later shipment to the sponsor for analysis of selected metabolites and bile acids. Any gallstones or gallsand detected during necropsy were stored, then shipped to the sponsor for analysis of selected metabolites and bile acids.

Organs weights: wts of the following organs were recorded: adrenal, brain, epididymis, heart, kidney, liver/gallbladder, lung, ovary, pituitary, prostate, salivary gland [mandibular], seminal vesicles, spleen, testis, thymus, thyroids/parathyroids, uterus/cervix.

Histopathology: the following tissues were examined microscopically in all animals [including those that died or were sacrificed prematurely]: adrenal, aorta, brain, cecum, cervix, colon, duodenum, epididymis, esophagus, eyes [Davidson's fixative], femur/bone marrow, heart, ileum, jejunum, kidney, gross lesions, liver/gallbladder, lung/mainstem bronchi, lymph node [maxillary, mesenteric], mammary gland, optic nerve, ovary, pancreas, pituitary, prostate, rectum, salivary gland [mandibular], sciatic nerve, seminal vesicle, skin, skeletal muscle [thigh], spinal cord [cervical, thoracic, lumbar], spleen, sternum/bone marrow, stomach, testis, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, vagina.

Gallstones/gallsand analysis: Gallstones [calculi] and gallsand [granular material] samples were collected and stored until shipped to the sponsor for analysis. According to the report, "Samples from selected animals were analyzed for selected metabolites... and for nondrug-related constituents". The analysis of gallstone, gallsand, and bile was conducted by ~~_____~~. Gallstone/gallsand analysis: gallstones and gallsand were examined in 2 phases. In Phase 1, the exterior surface and the internal core of gallstones and individual gallsand were examined using light microscopy and SEM; structural elements were further examined using "...appropriate microanalytical techniques such as energy dispersive x-ray spectrometry...for inorganic elements and infrared microspectroscopy...for organic phases". In Phase 2, bile acids were quantitated in gallstones and gallsand. Gallstones, gallsand, and bile samples were collected from 4, 6, and 21 animals, respectively; it was not stated in the methods section whether or not any combination of these was collected from individual animals or if samples were obtained from a total of 31 animals.

Toxicokinetics: blood samples were collected [from femoral vein] on Day 1 and during Wk 33, at 1, 2, 4, 8, and 24 hrs postdosing. In addition, samples were collected from HD animals on Day 5, at the same times postdosing. Blood samples were shipped to the sponsor for analysis of BMS-330739 and metabolites, BMS-337070, BMS-337044, BMS-337045, BMS-337047, and DCP. In addition, sulfate conjugates, DM-1458 and DM-1460 [of the hydroxy and dehydro hydroxy derivatives] were assayed in samples collected during Wk 33. Control samples were discarded without analysis.

Results:

Mortality: there were 2 unscheduled deaths. One HDF was sacrificed moribund during Wk 3 [#I06740] due to severe drug-related clinical signs [i.e., hypoactivity, hunched posture, tremors ("limbs-all and body-entire"), excessive salivation, recumbency (lateral, sternal), unusual posture, impaired motor activity, severely reduced food intake]; this animal was replaced [with #I06742]. One HDM was sacrificed moribund during Wk 20 due to severe diarrhea. The sponsor noted that, in this animal, "Clinical pathology findings were consistent with an incidental inflammatory bowel disorder..." and was not considered drug-related. Drug-related clinical signs [i.e., hypoactivity, hunched posture, tremors (all limbs, all body), excessive salivation, vomiting, and decreased food intake] were evident in this animal prior to sacrifice.

Clinical signs: drug-related clinical signs were evident at all doses. Clinical signs observed at all doses include hypoactivity, tremors [whole body], hunched posture [at LD only in females] and reduced food intake. The cessation of dosing in HD animals during Days 2-5 and the decrease in HD from 100 to 75 mg/kg was based on the severity of hypoactivity observed following the first 100-mg/kg dose. There were no clear drug-related effects on rectal temperature or respiratory rate.

Body weights: there were no significant effects on body wt. However, mean body wt tended to

be lower in HDM [compared to CM] from about Wk 30 on [7-13%]. Mean body wt gain was not notably affected. During Wks 30-40, mean body wt gain in HDM was similar to that in CM.

Food consumption: no quantitated. Qualitative differences discussed under "clinical signs".

Ophthalmoscopy: no drug-related findings reported.

ECG/bp/hr: there were no clear drug-related effects on bp. ECG and hr data were not summarized by the sponsor. According to the sponsor, there were no drug-related changes in ECG parameters or hr.

Hematology: there were no clear drug-related findings. During Wk 13, fibrinogen tended to be increased in MD [3-11%] and HD [6-22%] animals, and wbc and lymphocyte cts decreased in MDM [30-35%] and HDM [30-44%]; only the increase in lymphocyte ct was statistically significant. Lymphocyte ct also tended to be decreased during Wks 26 [MD (26%), HD (34%)] and 40 [26%].

Clinical chemistry: there were no clear drug-related findings. AST and ALT were each elevated in 1 HDM during Wk 40 [M I06719 (AST: 2.5-fold compared to high-CM value, 4-fold compared to mean CM value); ALT: M I06733 (2-fold compared to high-CM value, 3-fold compared to mean CM value)]. ALT was also elevated in M I06733 during Wks 13 [2-2.4 fold] and 26 [1.4-2.3 fold]; this animal's baseline value was similar to the high-CM baseline value and 1.4 fold (44%) higher than the mean CM value.

Urinalysis: there were no clear drug-related effects; urinary volume data were quite variable.

Organ weights: findings of note consisted of the following: (a) a decrease in seminal vesicle wt [absolute-relative] in LDM [17-15%], MDM [70-67%], and HDM [70-61%] and (b) a decrease in thyroid/parathyroid wt [absolute-relative] in HDF [43-40%].

Gross pathology: the sponsor noted that there were no drug-related gross findings in animals sacrificed prematurely. The only findings of note were detected in gallbladder [summarized below].

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
gallbladder	mucooid material	0/4	0/4	1/4	1/4	0/4	3/4	2/4	1/4
	granular material	0/4	1/4	1/4	1/4	0/4	1/4	3/4	1/4
	calculus	0/4	0/4	1/4	3/4	0/4	0/4	1/4	2/4

i.e., gallsand

Histopathology: there were no clear drug-related findings as presented in the summary table.

However, the sponsor noted that drug-related microscopic findings were detected in 1 MDM, 1 MDF, and 1 HDM. These included the following: (a) Kupffer cell hypertrophy and hyperplasia [characterized as minimal-slight], (b) minimal lymphohistiocytic periportal inflammation; some foci were accompanied by minimal vacuolar degeneration of hepatocytes [in the affected males], and (c) PAS-positive inclusions/concretions in Kupffer cells, interlobular bile ducts, and/or bile canaliculi. The liver findings [i.e., "focal hepatolithiasis"] were considered secondary to deposition of drug-related material in the biliary system and gallbladder. No evidence of cholestasis was detected.

Gallstone/gallsand analysis: Gallstones were characterized as follows:

"Morphologic evaluation of gallstones revealed that they were brittle and had a variably formed, dry, and compact outer shell and a soft, granular, yellow-brown homogenous core. The morphologic features of the gallsand were comparable to the inner core material of the gallstones."

The gallstones were found to contain "a mixture of aripiprazole metabolites" and bile acids [tauro bile acid salts, in particular]. The brownish-appearing areas contained more

bile-salts and less drug-related material, and *vice versa* in the yellowish-appearing areas. There was no evidence of Ca- or cholesterol-containing "structures".

"Semi-quantitative" analysis of gallsand and gallstones by _____ resulted in the detection of 6 Phase II metabolites of aripiprazole. Two sulfate conjugates [i.e., of hydroxy BMS-337039 (BMS-337042) and dehydro hydroxy BMS-337039 (BMS-511426)] were the primary drug-related compounds detected. According to the report text, these 2 conjugates accounted for 11-74% and 2-17% of gallsand and gallstones, respectively, by wt. Conjugates of 4 other hydroxy metabolites [3 sulfates, 1 glucuronide] were present in lesser amounts [$<4.3\%$]. The gallstones and gallsand samples also contained bile acids, taurodeoxycholic acid, in particular.

Toxicokinetics: the data were summarized in the following sponsor's tables [Tables 10-11]:

Table 10: Mean (SD) toxicokinetic values for BMS-337039 after daily doses of BMS-337039 in monkeys in study 99354

BMS-337039 (Aripiprazole)									
Dose mg/kg	Study Period	C _{MAX} (ng/mL)		T _{MAX} (h)		AUC** (ng·h/mL)			
		M	F	M	F	M	F		
25	Day 1	462 (361)	548 (359)	4.00* (2.00,4.00)	4.00* (2.00,4.00)	3743 (1731)	4655 (1904)		
	Week 33	789 (279)	876 (313)	4.00* (1.00,4.00)	4.00* (4.00,4.00)	7667 (3059)	8286 (2688)		
50	Day 1	1221 (364)	888 (592)	4.00* (4.00,8.00)	3.00* (1.00,4.00)	13413 (5293)	8889 (5340)		
	Week 33	1039 (381) _a	1765 (908)	4.00* (4.00,4.00) _a	4.00* (4.00,4.00)	9249 (4071) _a	14610 (7160)		
75	Day 1b	1301 (426)	848 (623)	2.00* (1.00,4.00)	1.50* (1.00,2.00)	13078 (4537)	7949 (4364)		
	Week 33	1501 (880)	2571 (1002)	4.00* (2.00,4.00)	4.00* (2.00,4.00)	20647 (13546)	23740 (6286)		
100	Day 1	1372 (359)	1126 (380)	3.00* (2.00,4.00)	2.00* (1.00,2.00)	13542 (4077)	12361 (3967)		

* Median (Minimum, Maximum); ** AUC=TAUC(0-T), trapezoidal area under the curve from time zero to time T, where T = 24 h; a: N = 3; and b: although the plasma samples were collected on day 5 (day 1 after the first 75 mg/kg dose), for the ease of presentation, this collection period was designated as day 1.

Table 11: Mean (SD) toxicokinetic values for BMS-337040, BMS-337044, BMS-337045, BMS-337047, and DCPD after daily doses of BMS-337039 in monkeys in study 99354

BMS-337040 (DM-1451)									
Dose mg/kg	Study Period	C _{MAX} (ng/mL)		T _{MAX} (h)		AUC** (ng·h/mL)			
		M	F	M	F	M	F		
25	Day 1	32.2 (31.8)	32.2 (13.1)	4.00* (2.00,8.00)	3.00* (2.00,4.00)	140 (106)	175 (87.7)		
	Week 33	29 (18.1)	23 (17.0)	6.00* (2.00,8.00)	2.00* (2.00,8.00)	296 (235)	199 (157)		
50	Day 1	135 (119)	133 (88.7)	2.00* (2.00,4.00)	2.00* (1.00,4.00)	913 (810)	878 (473)		
	Week 33	14.8 (11.0) _a	23.1 (20.0)	2.00* (1.00,8.00) _a	3.00* (2.00,4.00)	170 c	166 (138)		
75	Day 1b	43.9 (25.5)	29.3 (22.6)	1.50* (1.00,4.00)	1.00* (1.00,2.00)	290 (96.7)	270 (254)		
	Week 33	40.3 (30.4)	160 (56.2)	4.00* (2.00,4.00)	3.00* (2.00,4.00)	421 (240)	996 (204)		
100	Day 1	168 (56.8)	196 (93.4)	3.00* (1.00,4.00)	2.00* (2.00,8.00)	1315 (647)	1580 (893)		

BMS-337044 (OPC-14587)									
Dose mg/kg	Study Period	C _{MAX} (ng/mL)		T _{MAX} (h)		AUC** (ng·h/mL)			
		M	F	M	F	M	F	M	F
25	Day 1	264 (147)	282 (90.3)	4.00* (4.00,8.00)	6.00* (4.00,8.00)	3517 (1926)	3429 (1705)		
	Week 33	456 (163)	467 (208)	4.00* (2.00,8.00)	4.00* (2.00,4.00)	7347 (3506)	5821 (3638)		
50	Day 1	405 (99.1)	365 (144)	4.00* (2.00,4.00)	4.00* (2.00,8.00)	6736 (2488)	5880 (1922)		
	Week 33	633 (129) _a	756 (265)	4.00* (4.00,4.00) _a	4.00* (4.00,4.00)	8610 (4233) _a	10001 (4828)		
75	Day 1b	425 (64.9)	458 (60.7)	3.00* (2.00,24.0)	3.00* (2.00,4.00)	7626 (1879)	5798 (1297)		
	Week 33	727 (441)	863 (249)	8.00* (2.00,8.00)	4.00* (4.00,8.00)	12461 (7847)	10477 (2184)		
100	Day 1	389 (71.5)	451 (15)	14.0* (4.00,24.0)	4.00* (4.00,8.00)	6535 (2136)	6824 (443)		

BMS-337045 (DM-1452)									
Dose mg/kg	Study Period	C _{MAX} (ng/mL)		T _{MAX} (h)		AUC** (ng·h/mL)			
		M	F	M	F	M	F	M	F
25	Day 1	98.9 (66.8)	92.8 (41.1)	4.00* (4.00,8.00)	4.00* (2.00,4.00)	952 (427)	981 (331)		
	Week 33	185 (84.7)	219 (71.3)	4.00* (2.00,8.00)	4.00* (4.00,8.00)	1860 (862)	2592 (900)		
50	Day 1	144 (53.6)	184 (121)	4.00* (4.00,8.00)	3.00* (2.00,4.00)	1744 (622)	2317 (1151)		
	Week 33	233 (102) _a	294 (125)	4.00* (4.00,8.00) _a	4.00* (4.00,4.00)	2718 (1209) _a	3231 (1445)		
75	Day 1b	156 (52.7)	117 (39.2)	2.00* (2.00,4.00)	3.00* (2.00,4.00)	2185 (904)	1184 (443)		
	Week 33	248 (128)	378 (96.3)	6.00* (4.00,8.00)	6.00* (4.00,8.00)	3830 (1957)	4750 (1042)		
100	Day 1	172 (30.8)	157 (50.0)	4.00* (2.00,4.00)	2.00* (2.00,4.00)	2168 (455)	1845 (452)		

BMS-337047 (OPC-3373)									
Dose mg/kg	Study Period	C _{MAX} (ng/mL)		T _{MAX} (h)		AUC** (ng·h/mL)			
		M	F	M	F	M	F	M	F
25	Day 1	59.6 (65.8)	92 (45.3)	1.50* (1.00,4.00)	1.00* (1.00,2.00)	252 (270)	411 (240)		
	Week 33	28.3 (17.3)	37.8 (8.83)	8.00* (1.00,8.00)	4.00* (2.00,4.00)	349 (172)	371 (114)		
50	Day 1	124 (67.2)	183 (66.3)	2.00* (1.00,2.00)	1.50* (1.00,2.00)	696 (177)	813 (259)		
	Week 33	35.4 (5.65) _a	242 (267)	2.00* (1.00,2.00) _a	3.00* (1.00,4.00)	323 (175) _a	1375 (1532)		
75	Day 1b	188 (103)	219 (119)	1.00* (1.00,4.00)	1.50* (1.00,24.0)	1446 (1282)	1283 (197)		
	Week 33	175 (43.5)	402 (126)	4.00* (2.00,4.00)	3.00* (1.00,4.00)	1463 (495)	3061 (873)		
100	Day 1	210 (175)	299 (138)	2.00* (1.00,4.00)	2.00* (1.00,2.00)	2270 (1090)	2673 (2276)		

DCPP									
Dose mg/kg	Study Period	C _{MAX} (ng/mL)		T _{MAX} (h)		AUC** (ng·h/mL)			
		M	F	M	F	M	F	M	F
25	Day 1	42.1 (24.4)	109 (79.6)	4.00* (4.00,8.00)	4.00* (2.00,4.00)	375 (232)	764 (427)		
	Week 33	96 (61.4)	177 (67.8)	3.00* (2.00,4.00)	4.00* (2.00,8.00)	724 (318)	1807 (719)		
50	Day 1	94.7 (32.9)	91.1 (44.6)	4.00* (2.00,8.00)	3.00* (1.00,4.00)	943 (270)	876 (320)		
	Week 33	199 (162) _a	180 (104)	4.00* (2.00,4.00) _a	4.00* (2.00,4.00)	1284 (955) _a	1823 (1159)		
75	Day 1b	129 (50.1)	171 (79.5)	1.50* (1.00,2.00)	2.00* (2.00,2.00)	1504 (649)	1612 (846)		
	Week 33	184 (119)	344 (21.0)	8.00* (2.00,8.00)	6.00* (2.00,8.00)	2752 (1661)	4508 (457)		
100	Day 1	125 (59.4)	205 (119)	4.00* (1.00,4.00)	5.00* (1.00,8.00)	1453 (763)	2709 (1995)		

Summary of individual study findings

Acute: acute toxicity studies were conducted in ICR [CD-1] mouse [p.o.], Sprague-Dawley rat [p.o., i.v.] and cynomolgus monkey [p.o., i.v.]. In mouse, doses of 327-4000 mg/kg resulted in 100% mortality; 1/3 females died at 93 mg/kg. Clinical signs were evident at all doses [decreased activity, ptosis, prone position, cool to touch, convulsions]. Unabsorbed drug in the GI was detected in animals found dead. No gross findings were detected in survivors. In rat, oral dosing was associated with severe clinical signs [including convulsions] and death [LD₅₀ = 965 mg/kg in males, 705 mg/kg in females]. Gross findings in animals that died were detected in GI [unabsorbed drug, gastric mucosal hemorrhage] and adrenal gland [enlargement]. In monkey, oral dosing was associated with CNS signs [all doses (500-200 mg/kg)] and

adverse effect on body wt and food consumption, but there were no drug-related deaths. Histopathology was not performed in any of the oral studies.

The doses used in the i.v. toxicity studies in rat [0, 0.1-0.4, 2 mg/kg] and monkey [0, 0.05, 0.2, 1 mg/kg] were not associated with death or any effect on any parameter assessed [including histopathology], indicating that sufficiently high doses were not used in either study.

Subchronic: subchronic toxicity studies were conducted in Sprague-Dawley rat [13-wk + 4-wk recovery, 4-wk + 4-wk recovery, 5-wk "screening"] and cynomolgus monkey [4-wk dose-ranging, 13-wk + 4-wk recovery].

In the 13-wk [+ 4-wk recovery] oral [gavage] study in rat [0, 6, and 20 mg/kg], no unscheduled deaths occurred and no drug-related clinical signs were evident. There was a slight effect on body wt in males at 20 mg/kg; the final mean body wt at that dose was 6% lower compared to CM. Body wt was increased in females at 6 and 20 mg/kg, with final body wts being 13 and 8% higher, respectively, compared to CF. Slight increases in hgb and hct were noted in males at 6 and 20 mg/kg. TG levels were decreased in males and phospholipids were increased in females at 20 mg/kg. No drug-related findings were detected on urinalysis parameters. Organ wt findings consisted of a small decrease in liver wt in males at 20 mg/kg, and decreases in ovary and uterus wt in females at 6 and 20 mg/kg. Microscopic findings were detected only in females, i.e., mammary gland lobular hyperplasia at 6 and 20 mg/kg, acinar dilatation and secretion at 20 mg/kg. Vaginal mucification was detected at 6 and 20 mg/kg. The lack of dose-limiting toxicities in this study suggests that higher doses would have been tolerated.

Shorter-term studies were conducted at higher doses. In a 4-wk [+ 4-wk recovery] study in rat, doses of 0, 60, and 100 mg/kg were administered by gavage. No unscheduled deaths occurred. Sedation was observed at both doses. Additional clinical signs at the 100 mg/kg consisted of abdominal wetness, lacrimation, hypothermia [1/10 M, 2/10 F], and tremors. Body wt effects were evident at both doses in males and females. Final body wts were 19 and 34% lower in males at 60 and 100 mg/kg, respectively, compared to CM, and 15 and 25% lower in females at 60 and 100 mg/kg, respectively, compared to CF. Food consumption was reduced, consistent with effects on body wt. No drug-related ophthalmology findings were detected. Numerous hematology parameters were affected. In females, decreases in MCV, platelet ct, and reticulocytes were observed at both doses. In males, decreases in MCV, platelet ct, wbc ct, basophil ct, LUC ct, and in coagulation parameters [PT, APTT] were observed at 100 mg/kg; platelet ct, and PT and APTT were also reduced at 60 mg/kg. Evidence of bone marrow cytotoxicity was evident upon examination of marrow smears at both doses in males and females, i.e., decreases in nucleated cells, proerythroblasts/orthochromatic erythroblasts, and neutrocytes. Decreases in megakaryocytes and mitotic cells were evident at one or both doses only in males. Bone marrow cytotoxicity was confirmed upon microscopic examination. Hypocellularity of bone marrow was detected in males at 100 mg/kg, and in females at both 60 and 100-mg/kg.

Numerous clinical chemistry findings were also noted. Primary findings consisted of increases in LDH at 60 mg/kg, and in GTP, SGOT, and SGPT in females at both doses; the increases in SGOT and SGPT were particularly marked. No microscopic correlates for these findings were detected upon examination of liver. Serum lipids were reduced in females at 100 mg/kg; only TG were reduced in males [both doses]. Serum glucose and Ca were decreased in males at both doses. Serum Ca was also reduced in females at both doses, but glucose was not affected. Decreases in selected globulins [α , β] and total globulins were decreased in males at 100 mg/kg; α_1 -globulin was reduced in females at 100 mg/kg. Small increases in Cl and K were noted in males at 100 mg/kg. Water consumption was significantly reduced at both doses in males and females, consistent with decreases in food consumption. Urine volume and urinary electrolyte concentrations and creatinine were also reduced at both doses in males and females. Adrenal gland wt was increased in males and females at both doses. Pituitary [both doses] and ovary [100

mg/kg] wts were reduced in females. Seminal vesicle [100 mg/kg] and prostate [both doses] wts were reduced in males. Microscopic findings were detected in adrenal gland [hypertrophy of the z. fasciculata/reticularis], pituitary gland [atrophy of the pars intermedia], lung [foamy cells], mammary gland [lobular hyperplasia, milk secretion], bone marrow [hypocellularity], submaxillary and sublingual glands [hypertrophy of acinar cells], prostate [flattening of glandular epithelium, decreased secretion], ovary [decreased CL], uterus [atrophy], and vagina [mucification]. Adrenal gland, pituitary gland, and lung were affected in males and females at both doses. As noted previously, bone marrow was affected in males and females at 100 mg/kg, and in females at 60 mg/kg. Mammary gland was affected only in females. The submaxillary and sublingual glands were affected at both doses, but only in males. Female reproductive organs were affected at both doses, except for vaginal mucification [100 mg/kg only]. The sponsor considered most of the drug-related findings, including the bone marrow and adrenal gland effects, to be secondary to adverse effects on body wt and food consumption. Atrophy of the pituitary pars intermedia was considered a direct D₂ agonist effect. Drug-related microscopic findings were not detected in recovery animals.

In a 5-wk screening study in rat, doses of 2, 6, 20, and 60 mg/kg were administered by gavage. There were no unscheduled deaths or drug-related clinical signs at doses <60 mg/kg. At 60 mg/kg, sedation, lacrimation, piloerection, tremor, and "loss of vigor" were evident. Body wt and food consumption were adversely affected only at 60 mg/kg; final body wts were 19-17% lower in males and females at 60 mg/kg compared to C grps. Clinical pathology findings were observed primarily at 60 mg/kg. Most notable were increases in SGOT and creatinine in females and decreases in glucose and Ca in both males and females. No hematology findings were noted except for small decreases in MCV and MCH in females at 60 mg/kg. [Urinalysis parameters were not assessed.] Organ wt findings included a small decrease in liver wt in males at 60 mg/kg, increases in relative adrenal gland wt [males and females] and a decrease in ovary wt at 60 mg/kg. Microscopic findings were detected in liver [atrophy], spleen [atrophy, white pulp], bone marrow [vacuolar degeneration, decreased hematopoiesis], ovary [degeneration of granulose cells], and uterus [endometrial atrophy]. Ovary, uterus, and spleen findings were observed only at 60 mg/kg. Bone marrow effects were detected at 20 and 60 mg/kg, and in 1/8 females at 6 mg/kg. Hepatocellular atrophy was observed only at 60 mg/kg in males, but at doses of 6, 20, and 60 mg/kg in females [only 1/8 was affected at 6 mg/kg].

In monkey, only the 13-wk study was definitive; the 4-wk study was conducted in only 1/sex/grp. Doses of 0, 0.5, 1, 5, and 25 mg/kg were used in the 13-wk study. Clinical signs were observed at all doses, although only 1 low-dose animal was affected. Clinical signs observed at 5 and 25 mg/kg included decreases in spontaneous motor activity and catalepsy; ptosis was noted only at 25 mg/kg. There were no other drug-related findings. [According to the sponsor's NDA Toxicology summary, "moderate to severe muddy substance" was detected in bile at 25 mg/kg. Also, "gall sand" was detected in liver of 2/2 animals at 25 and 125 mg/kg in a preliminary 4-wk study in cynomolgus monkey.]

Chronic: chronic oral toxicity studies were conducted in Sprague-Dawley rat [52-wk, 26-wk + 13-wk recovery] and cynomolgus monkey [52-wk, 39-wk].

In rat, doses of 0, 1, 3, and 10 mg/kg were used in the 52-wk study. There were no drug-related deaths or clinical signs. Body wt was not adversely affected in either males or females. Clinical pathology parameters were minimally affected. Organ wt changes consisted of increases in ovary wt [3 and 10 mg/kg] and decreases in uterus wt [3 and 10 mg/kg] and liver wt [10 mg/kg in males]. Drug-related microscopic findings were observed only in females [lobular hyperplasia and milk secretion in mammary gland, uterine atrophy, and mucification of the vaginal epithelium]. Mammary gland findings were detected at all doses.

In the 26-wk + 13-wk recovery study, doses of 0, 10, 30, and 60 mg/kg were administered. None of the unscheduled deaths were considered drug-related by the sponsor. Drug-related clinical signs [hypo-, hyperactivity, ptosis] were evident at 30 and 60 mg/kg. Body wt was reduced [compared to Cs] at all doses in males and at 30 and 60 mg/kg in females, but increased in females at 10 mg/kg. In males, final body wts were 8, 21, and 39% lower compared to CM at 10, 30, and 60 mg/kg, respectively. In females, final body wt was 8% higher at 10 mg/kg and 9 and 24% lower at 30 and 60 mg/kg, respectively, compared to CF. Body wts were still reduced at the end of the recovery period in treated grps [25 and 17% in males and females, respectively]. Changes in food and water intake were fairly consistent with the body wt effects. There were no ophthalmology findings. The primary hematology finding was a decrease in wbc ct in males at 60 mg/kg; the decrease in total wbcs was due to decreases in neutrophils, lymphocytes, monocytes, eosinophils, and basophils. On coagulation parameters, PT was increased in males at 60 mg/kg, and at all doses in females. Primary effects on clinical chemistry parameters consisted of decreases in serum Ca [all doses, males and females], increases in P, and BUN at all doses in females and at 60 mg/kg in males. Decreases in serum lipids [TG, cholesterol] and glucose at all doses [except that TG was decreased only at 60 mg/kg in males]. In males, total protein, globulin, and albumin were reduced at 60 mg/kg. Urine volume was reduced at 30 and 60 mg/kg in males, and at all doses in females; however, the effect was not dose-related in females. Urinary pH was reduced at 30 and 60 mg/kg in males, and at 60 mg/kg in females. The sponsor considered many of the clinical pathology findings to be secondary to reduced body wt and food/water consumption. Primary effects on organ wts consisted of increases in adrenal, lung, and testis wts at 60 mg/kg. Uterus wt was reduced at all doses, but not in a dose-related manner. There were numerous microscopic findings. The following were observed [for the most part] only at 60 mg/kg: ventricular necrosis [in 1 male] and subacute inflammation [males] in heart, liver necrosis [in 2 males], chronic inflammation and/or necrosis of the tail [males and females], bilateral testicular atrophy, increased spermatogenic epithelium in epididymes, and retinal degeneration [1 male, 2 females]. An increase in the severity of lipofuscin pigment deposition was noted in adrenal gland [60 mg/kg in males, 30 and 60 mg/kg in females] and ovary [60 mg/kg]. Subchronic inflammation of the prostate [ampullary gland] was increased at 30 and 60 mg/kg. Atrophy of the pars intermedia of the pituitary gland was detected at all doses in males and females; the severity of the effect was increased in males at 60 mg/kg. Female reproductive organs and mammary gland [in females] were affected at all doses. Mammary gland effects were consistent with stimulation, whereas the other reproductive effects reflected a more quiescent state. However, the increase in lipofuscin pigment in ovary [also in adrenal cortex] may be related to other effects. Interestingly, ketoconazol, an antifungal drug, was found to increase lipofuscin pigment deposition in ovary and adrenal gland, a possible mechanism being inhibition of steroid synthesis [Greaves P. (2000) *Histopathology of Preclinical Toxicity Studies* 2nd Ed, pg 718, Elsevier]. Lung histiocytosis was observed at 30 and 60 mg/kg in both males and females, with the severity being greater in females at the highest dose. In recovery animals, findings were still detected in lung [histiocytosis], adrenal cortex [lipofuscin pigment (increased severity)], and ovary [lipofuscin pigment (increased severity)].

In the 52-wk study in monkey, aripiprazole was administered by gavage at doses of 0, 0.5, 5, and 25 mg/kg. There were no unscheduled deaths. Drug-related clinical signs [i.e., tremors, catalepsy, impaired motor activity, hyporeactivity, abnormal posture] were evident at doses of 5 and 25 mg/kg. Transient body wt loss [reflected in reduced food intake] was observed at 25 mg/kg; however, final body wts were similar among grps. No drug-related effects were observed on ophthalmologic, ECG, or clinical pathology parameters. Absolute organ wts were not affected; however, dose-corrected wts of male and reproductive organs were reduced at 5 and/or 25 mg/kg. There were no clear drug-related microscopic findings. According to the sponsor's NDA Toxicology summary, gallsand and gallstones were observed at 25 mg/kg; however, there was no evidence of "hepatobiliary disease".

In the 39-wk study in monkey, aripiprazole was administered at higher doses [0, 25, 50, 100/75 mg/kg]. The initial high dose [100 mg/kg] was not tolerated, as evidenced by severe hypoactivity following the

first dose. High-dose animals received no doses on Day 2-4. On Day 5, the high dose was lowered to 75 mg/kg. It was noted that nasal gavage was used when necessary [due to resistance or vomiting]. Two high-dose animals were sacrificed moribund during Wks 3 and 20 due to severe drug-related clinical signs [i.e., hypoactivity, hunched/unusual posture, tremors (whole-body), excessive salivation, recumbency, impaired motor activity, vomiting]. [Evidence of inflammatory bowel disorder was detected in 1 of these animals; however, the sponsor didn't consider this finding drug-related.] Clinical signs [i.e., hypoactivity, tremors (whole body), hunched posture, reduced food intake] were evident at all doses. There were no significant effects on body wt, upon ophthalmology examination, or on clinical pathology parameters. Organ wt effects consisted of decreases in seminal vesicle wt at all doses and a decrease in thyroid wt in females at 75 mg/kg. The only gross finding detected was material [mucoid, granular, calculus] in the gallbladder; the incidence of calculi was dose-related in males and females [50 and 75 mg/kg]. There were no clear dose-related increases in microscopic findings. However, the sponsor considered findings in liver [indicative of "focal hepatolithiasis"] in 1 male and 1 female at 50 mg/kg and 1 male at 75 mg/kg to be drug-related. These consisted of Kupffer cell hypertrophy and hyperplasia, minimal lymphohistiocytic periportal inflammation [with some foci being accompanied by minimal vacuolar degeneration of hepatocytes], and PAS-positive inclusions/concretions in Kupffer cells, interlobular bile ducts, and/or bile canaliculi. There was no evidence of cholestasis. The sponsor referred to the material deposited in the bile duct and gallbladder as gallstones or "gallsand". This material was further characterized as being "brittle" with a "variably formed, dry, and compact outer shell and a soft, granular, yellow-brown homogenous core". The material making up the gallsand was stated to be similar to the material in the inner core of the gallstones. Upon biochemical analysis, the material was found to consist of "a mixture of aripiprazole metabolites" and bile acids [taurodeoxycholic acid, in particular]. There was no evidence of Ca- or cholesterol-containing material. Six conjugated metabolites of aripiprazole were detected, with sulfate conjugates, BMS-337042 and BMS-511426, being primary [accounting for 11-74 and 2-17% of gallstone and gallsand material, respectively]. An analysis of bile collected at the end of the study indicated the presence of 3 conjugated metabolites, BMS-337041 [glucuronide conjugate of DM-1451], BMS-337042 [sulfate conjugate of DM-1451], and BMS-511426 [sulfate conjugate of DM-1459]. The concentration of BMS-337042 in bile was greater than that of the other 2 conjugates, and the concentration of BMS-337041 was somewhat greater than that of BMS-511426. Although BMS-337041 was present in bile at relatively high levels, the low amount of this metabolite in gallstones and gallsand [≈2% by weight] is consistent with its greater solubility [10 times that of the other conjugates, as determined *in vitro*]. Concentrations of BMS-337042 and BMS-511426 in bile exceeded the solubility limits determined *in vitro*.

Toxicology Summary and Conclusions

The definitive toxicity studies consisted of 13-wk + 4-wk recovery, 4-wk + 4-wk recovery, 52-wk, and a 26-wk + 13-wk recovery oral toxicity studies in Sprague-Dawley rat and 13-wk + 4-wk recovery, 52- and 39-wk oral toxicity studies in cynomolgus monkey. Testing was adequate based on clear dose-limiting toxicities in both species. In the chronic toxicity studies, only the CNS [based on clinical signs] was a common target organ in both in rat and monkey.

In rat, a number of target organs were identified. CNS-related clinical signs [e.g., hypoactivity, sedation] were evident at higher doses. There was some indication of bone marrow effects [e.g., suppression]; however, these were not consistently observed. Although different dose ranges were used, the 60-mg/kg dose was included in all but the 13-wk study and was associated with bone marrow effects in the short-term studies. In the 4-wk study, decreases in hematological parameters [e.g., wbc ct, platelet ct, reticulocytes] were correlated with significant decreases in cellular elements in bone marrow smears [e.g., nucleated cells, proerythroblasts/orthochromatic erythroblasts, megakaryocytes, mitotic cells] and hypocellularity of the bone marrow upon microscopic examination. In a 5-wk screening study, vacuolar degeneration [severe in 1 HDF] and decreased hematopoiesis were detected in bone marrow upon

microscopic examination. Hematological parameters were not notably affected, but atrophy of the white pulp was detected in spleen. In the 26-wk study, wbc's [neutrophils, lymphocytes, monocytes, eosinophils, basophils] were reduced, but no microscopic changes in bone marrow were evident [bone marrow smears were not examined]. The sponsor attributed the bone marrow effects to decreases in body wt and food consumption. Certainly bone marrow suppression may result from such decreases; however, similar body wt effects were observed in the 4- and 26-wk studies, and bone marrow was not affected in the 26-wk study. It is notable that in the 4-wk study, the bone marrow hypocellularity was characterized as slight and was not observed in animals examined after a 4-wk recovery period. Also, bone marrow effects were not observed at doses up to 60 mg/kg in a 2-yr oral carcinogenicity study.

A number of effects observed in the rat toxicity studies may have been secondary to the body wt/food consumption effects, including effects on water consumption and related urinary findings, serum glucose, absolute organ wts, and some microscopic findings. Hypertrophy of the adrenal cortex [z. fasciculata, z. reticularis] and atrophy of male reproductive organs [e.g., testis] may result from decreases in body wt/food consumption or, alternatively, from alterations in hormonal balance as well as a direct toxic drug effect. [Adrenal gland effects are discussed in the summary of the carcinogenicity study data.] With aripiprazole, it is difficult to determine the mechanism(s) underlying these changes since body wt/food consumption and/or hormonal changes [e.g., prolactin] may be involved. Microscopic changes observed in mammary gland [in females] and in female reproductive organs are consistent with elevations in serum prolactin. Although serum prolactin levels were not measured in the general toxicology studies, levels of serum prolactin were demonstrated to be elevated in Sprague-Dawley rats in a series of special studies. In a 13-wk study, serum prolactin was increased at all doses, however, the effects were significant only at the lower doses. Another interesting observation in that study was that, consistent with the serum prolactin data, the incidence of diestrus exhibited an inverse U-shaped function and was not significantly increased at the HD [60 mg/kg]. In contrast, the effect on mammary gland was clearly dose-related. Although hyperplasia was detected in the majority of females at all doses, mammary gland distension with secretion was significantly increased only at 60 mg/kg. The sponsor attributed the serum prolactin effect to D₂ antagonist effects at the lower doses and D₂ partial agonist effects at the high dose; however, the mammary gland effects, consistent with hyperprolactinemia, were not consistent with D₂ partial agonist activity at the high-dose.

Atrophy of the pars intermedia of the pituitary gland, observed in both males and females was considered by the sponsor to be a result of D₂ agonist activity. The sponsor noted that bromocriptine, a D₂ agonist, exerts a similar effect. This effect, however, is in contrast to D₂-antagonist-like effects on serum prolactin as evidenced by the dose-related increase in mammary gland secretion in females. Effects on male reproductive organs are consistent with an elevation in serum prolactin [and are similar to those observed in the male fertility study and the carcinogenicity study in Sprague-Dawley rat]; however, in the 13-wk special study, serum prolactin was significantly reduced at the 60-mg/kg dose. Therefore, the data represent a somewhat inconsistent pattern, both with respect to microscopic finding and changes in serum prolactin and with respect to specific microscopic findings.

Other target organs included lung and eye. The lung finding [foamy macrophages] was characterized [using electron microscopy] as appearing similar to "spontaneous pulmonary histiocytosis". However, the incidences of this finding were clearly dose-related in both studies in which it was detected; in addition, it was still evident in recovery animals in the 26-wk study. The sponsor characterized this finding as drug-induced phospholipidosis, based on EM examination of lung tissue. Lung histiocytosis was also a drug-related finding in the carcinogenicity study in Sprague-Dawley rat. The severity of the finding was increased at 40 and 60 mg/kg. Retinal degeneration was detected only in the 26-wk toxicity study, and only in a few animals at the high-dose. The finding was not observed in recovery animals; however, this should not be considered evidence of recovery since only a few main-study animals were affected and only 5/sex C and HD animals were followed during the recovery period. Bilateral retinal degeneration [of

moderate severity in a number of affected animals] was observed in the Sprague-Dawley rat carcinogenicity study. The incidence was increased at 40 and 60 mg/kg, and greater in females than in males. The sponsor noted that degeneration was detected in the "outer nuclear layer and photoreceptor cell layer most severely", and were similar to changes observed with light-induced retinopathy. Ocular toxicity has been observed following administration of other antipsychotic drugs. For example, phenothiazines and, more recently, seroquel have been associated with the formation of cataracts. Phenothiazines [e.g., chlorpromazine, thioridazine] have been reported to induce a retinopathy resulting from an interaction with melanin to produce pigment deposits in cornea, lens, and retina [Potts AM. Toxic responses of the eye. In: *Casarett and Doull's Toxicology: The Basic Science of Poisons*. Amdur MO et al. (Eds). New York: Pergamon Press, Inc., 4th Ed., pg. 521-562, 1991]. However, Leblanc et al. [Leblanc B et al. *Regul Toxicol Pharmacol* 28:124-132, 1998] recently concluded that "Careful review of the literature show...that a causal relationship between [retinal toxicity and melanin binding] has not been established" and phenothiazines [among other drugs] "exert adverse effects on nonpigmented ocular structures (cornea, lens), as a consequence of deposits of drug-related material". Regardless, aripiprazole induced retinal degeneration in nonpigmented rats; there was no mention of any deposition. A more relevant example of drug-induced retinal degeneration may be that observed in Wistar rats treated with pramipexole [a D₂ agonist]. Pramipexole [Mirapex[®], marketed for treatment of Parkinson's disease] produced retinal degeneration in a 2-yr carcinogenicity study; the finding was detected in Wk 76 of the study in animals dying or sacrificed prematurely. Retinal degeneration was not observed in a 1-yr chronic toxicity study. According to the Pharmacology/Toxicology Review of _____ [Thomas D. Steele, Ph.D., 12/14/95], the lesion observed in the carcinogenicity study was characterized as follows:

"The degeneration was characterized microscopically by a bilateral loss of photoreceptor (PR) cells. In about two-thirds of the affected animals, the lesion was characterized as 'mild'; it was restricted to the upper hemisphere of the eye, and the peripheral (outer) portion of the retina. In the 'severe' form (remaining one-third of affected animals), PR cell loss occurred in both hemispheres, the entire retina was lesioned, and vessels penetrated the retinal pigment epithelium..."

In the case of pramipexole, the sponsor conducted additional mechanistic studies to test the hypothesis that the retinal degeneration was due to a drug-induced "inhibition of the circadian-controlled disk-shedding/renewal system, which is necessary for a constant supply of 'fresh' disks in the PR cell....The disk-shedding/renewal system is apparently activated shortly after light onset by melatonin. The biosynthesis of melatonin in vertebrate retina is inhibited by D₂ receptor activation. Thus, stimulation of D₂ receptors by pramipexole prevents melatonin-induced activation of disk-shedding leading to retinal degeneration". Based on the results of the data from these studies, Dr. Steele concluded that:

"The data support the sponsor's contention that: 1) pramipexole-induced inhibition of disk-shedding/renewal is a **possible** mechanism of degeneration, and 2) non-pigmented animals are more susceptible to the damaging effects of pramipexole than pigmented animals. The latter hypothesis is the basis for the sponsor's assertion that the risk of retinal damage in humans is minimized by pigmentation. However, some outstanding deficiencies in experimental support for this hypothesis remain..."

Dr. Steele pointed out the following deficiencies [among others]: (a) there was no data to demonstrate that D₂ antagonists prevented the lesion, or that other D₂ agonists produced the lesion, (b) the sponsor cited a published study reporting that bromocriptine [a D₂ agonist] "**protected** against light-induced retinal damage, (c), no data were provided addressing the mechanism by which pigmentation was proposed to protect against pramipexole-induced retinal degeneration. The sponsor's panel of experts concluded that, since disk-shedding is a process present in all vertebrates [including humans], a risk to human retina could not be excluded. The retinopathy caused by pramipexole is included in labeling.

Aripiprazole is similar to pramipexole in that retinal degeneration was not observed in the carcinogenicity studies in albino mice [CD-1 (ICR)] or in the toxicity studies in cynomolgus monkey. However, retinal degeneration was evident in a few animals at the high-dose in the 26-wk study of aripiprazole, whereas retinal degeneration was not detected in a 1-yr toxicity study in rat with pramipexole. The retinal degeneration observed with aripiprazole was not addressed in any detail by the sponsor. It is not clear that the lesion observed with pramipexole is the same as that detected in rats treated with aripiprazole; however, bilateral injury was observed with both compounds [with aripiprazole, the incidence of unilateral retinal degeneration was also increased in males, particularly at 60 mg/kg in the 2-yr study]. The lesion appeared somewhat more severe with pramipexole; however, it is difficult to make such comparisons between studies. The NOEL for retinal degeneration is 20 mg/kg. This dose is 6.5 times the maximum recommended human dose on a mg/m^2 basis. The C_{max} and AUC for aripiprazole at this dose are 8 and 3 times, respectively, the C_{max} and AUC at the MRHD. Comparing this safety margin to that for pramipexole, according to the information provided in labeling, the AUCs at the effect doses for pramipexole are 2.5 and 12.5 times the AUC at the clinical dose; the AUC at the no-effect dose is 0.3 times the AUC at the clinical dose. The no-effect dose of pramipexole in rats is 0.6 times the clinical dose [1.5 mg t.i.d.] on a mg/m^2 basis. Therefore, the safety margin [based on plasma AUC for parent compound] is greater for aripiprazole than for pramipexole. However, aripiprazole is metabolized by CYP3A4 and CYP2D6. According to Dr. Hong's review of the human PK data [cf. Office of Clinical Pharmacology and Biopharmaceutics Review, Hong Zhao, Ph.D., HFD-860], plasma levels of the parent compound are increased 85% in CYP2D6 poor metabolizers. Therefore, the safety margin based on mean AUC data [for parent compound] may be an overestimate. Based on the uncertainty of the magnitude of the safety margin and the fact that a (possibly) similar lesion was detected with another D_2 agonist, it would seem prudent to ask the sponsor to investigate the possible mechanism(s) underlying the retinal degeneration observed in rats in order to allow a better assessment of the risk to humans.

Overall, the low-dose used in the 52-wk oral toxicity study could be considered a NOEL for male rats. [There was no NOEL established in female rats due to effects on mammary gland and female reproductive organs at all doses.] However, in neither males nor females were notable toxicities observed at doses up to 10 mg/kg in either the 52-wk or 26-wk studies; therefore, this dose could be considered a NOAEL in male and female rat. This dose is ≈ 3 times the MRHD on a mg/m^2 basis. The C_{max} and AUC for aripiprazole at this dose were 0.01-0.8 and 0.03-0.5 times, respectively, the C_{max} and AUC expected at the MRHD. [The plasma exposure estimated for the 52-wk study was notably lower than that quantitated in the 26-wk study.] Plasma exposure to OPC-14857 [the major, active metabolite] in humans was reported to be 40% that of the parent compound. In rat, the AUC for OPC-14857 was 8-17% of the AUC for aripiprazole.

In cynomolgus monkey, other than CNS, there were no direct target organs identified. The primary effects observed were severe clinical signs [e.g., hypoactivity, whole limb and/or body tremors] and deposition of apparent drug-related material in Kupffer cells and in bile duct and gallbladder. In the 39-wk study, the latter finding was not dose-related upon microscopic analysis. There was, however, a dose-related incidence of calculi in the gallbladder in males and females upon gross examination. Similar findings were detected in subchronic studies. In a preliminary 4-wk study, "gallsand" was detected in the liver of 2/2 animals at 25 and 125 mg/kg. In the 13-wk + 4-wk study, "moderate to severe muddy material" was detected at 25 mg/kg, but not at lower doses [0.5-5 mg/kg p.o.]. Analysis of the gallstones and gallsand from samples collected during the 39-wk study indicated the presence of 6 conjugated metabolites of aripiprazole, with sulfate conjugates, BMS-337042 and BMS-511426, being the most abundant; levels of these two conjugates in bile exceeded *in vitro* solubility limits. BMS-337042 and BMS-511426 were also detected in plasma, although at levels lower than parent compound [metabolite-to-parent ratios of 0.15-0.02]. In a separate study, bile samples collected from 2 animals administered 2-3

doses of aripiprazole [500-2000 mg/kg p.o.] confirmed the presence of BMS-337042. Similar liver, bile, and/or gallbladder findings were not observed in mouse or rat, consistent with the limited extent of metabolism to sulfate conjugates, particularly BMS-337042 and BMS-511426. [BMS-337042 and BMS-511426 were not detected in mouse plasma or "most" bile samples, and concentrations of these metabolites were close to or below the LLOQ in rat bile. Concentrations of BMS-337041 in mouse and rat bile were \approx 5-10 times lower than that in monkey bile.] According to the review of the human PK/ADME data [Office of Clinical Pharmacology and Biopharmaceutics Review, Hong Zhao, Ph.D., HFD-860], the sponsor demonstrated that concentrations of BMS-337041, BMS-337042, and BMS-511426 in human bile following repeat oral dosing [30 mg, 7 days] was "no more than 6% of the lowest bile concentrations found in the monkeys...and are well below...their limits of *in vitro* solubility". Therefore, the liver and gallbladder findings in monkey are probably not relevant to humans. The low dose used in the 52-wk study could be considered a NOEL in monkey. Although tremors were observed in one animal at that dose in the 13-wk study, no clinical signs were noted at that dose in the 52-wk study. The doses in the 39-wk toxicity study were \approx 16-50 times the maximum recommended human dose [MRHD] on a mg/m² basis. The plasma exposure [for aripiprazole] in monkeys at these doses are \approx 2, 2-4, and 5-6 [C_{max}] and 1, 1.2-2, and \approx 3 times [AUC] at 25, 50, and 75 mg/kg, respectively, the plasma exposure at the MRHD. The plasma AUC for the active metabolite, OPC-14857 [BMS-337044], is 40-95% of the AUC for aripiprazole [depending upon dose]. In humans, plasma levels of OPC-14857 are \approx 40% those of aripiprazole. However, as noted previously, safety margins for exposure based on mean AUC data for the parent compound in humans may be overestimates due to the possibility that higher plasma levels of parent compound may be achieved in poor metabolizers.

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V. GENETIC TOXICOLOGY

1. **Study title:** Mutagenicity study of OPC-14597 by bacterial DNA repair assay [Study no: 005552, Volume #1.65, Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceuticals, Ltd., Japan, Date of study initiation: 5/26/89, GLP, QA'd report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 8K84M — [CoA provided]

Formulation/vehicle: solution/DMSO

Methods

Strains/species/cell line: *bacillus subtilis* [strains H17 Rec⁺ and M45 Rec⁻]

Dose selection criteria

Basis of dose selection: n/s

Range finding studies: n/s

Test agent stability: reported to be stable in DMSO at 5% [HC]; a 10% solution was found not to be stable.

Metabolic activation system: n/a

Controls

Vehicle: DMSO

Negative controls: DMSO, kanamycin sulfate, distilled water

Positive controls: Mitomycin C

Exposure conditions

Incubation and sampling times: 24 hrs at 4° C followed by ≈20 hrs at 37° C.

Concentrations used in definitive study: 0.2, 2, 20, 200, 2000 µg/disc

Analysis:

No. of replicates: duplicates

Counting method: n/s

Criteria for positive results: a difference in length between the 2 strains of ≥3 mm. A positive result has to be confirmed "...in more than 2 experimental cases..."

Results

No inhibition of growth was detected in either tester strain treated with aripiprazole. Kanamycin sulfate inhibited growth of both tester strains equally. Mitomycin C inhibited growth of both tester strains, but had a greater inhibitory effect on M45 than on H17; a positive response was obtained in duplicate cultures.

2. **Study title:** Mutagenicity study of OPC-14597 by a bacterial reverse mutation test [Study no: 005551, Volume #1.65, Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Japan, Date of study initiation: 5/26/89, no GLP statement, QA'd report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 8K84M, — [CoA provided]

Formulation/vehicle: solution/DMSO

Methods

Strains/species/cell line: TA1535, TA1537, TA100, TA98, *E. coli* WP2uvrA

Dose selection criteria

Basis of dose selection: dose-range finding study

Range finding study: all tester strains; 50-5000 µg/plate

Test agent stability: stated to be "Stable on analysis after the study". Data provided indicated stability in DMSO for 6 hrs stored at ambient temperature and "scattering light".

Metabolic activation system: Sprague-Dawley rat liver S9, phenobarbital and 5,6-bezoflavone-induced.

Controls

Vehicle: DMSO

Negative controls: DMSO

Positive controls: N-ethyl-N'-nitro-N-nitrosoguanidine [ENNG] [TA1535], ICR-191 [TA1537], and furylfuramide [TA100, *E. coli* WP2uvrA]

in the absence of S9; 2-aminoanthracene in the presence of S9.

Comments: there was no indication that the S9 batch used was characterized with an agent other than 2-AA.

Exposure conditions

Incubation and sampling times: preincubation method used. Cultures were preincubated with drug for 20 min at 37° C, followed by incubation at 37° C for 48 hrs

Doses used in definitive study:

TA1535: 2.44, 4.88, 9.75, 19.5, 39, 78, and 156 µg/plate [-S9]; 4.88, 9.75, 19.5, 39, 78, 156, 313 µg/plate [+S9]

TA1537: 2.44, 4.88, 9.75, 19.5, 39, and 78 µg/plate [-S9]; 4.88, 9.75, 19.5, 39, 78, 156 µg/plate [+S9]

TA98: 4.88, 9.75, 19.5, 39, 78, and 156 µg/plate [-S9]; 9.75, 19.5, 39, 78, 156, and 313 µg/plate [+S9]

TA100: 1.56, 3.13, 6.25, 12.5, 25, and 50 µg/plate [-S9]; 3.13, 6.25, 12.5, 25, 50, and 100 µg/plate [+S9]

WP2uvrA: 39, 78, 156, 313, 625, 1250, 2500, and 5000 µg/plate [+/- S9]

Analysis

No. of replicates: duplicates in dose-range finding study; triplicates for the definitive study

Counting method: manual colony counter

Criteria for positive results: (a) a >2-fold increase in mean revertants at any concentration, (b) concentration-response relationship, (c) reproducible in 2 independent tests.

Results

Range-finding study: thin lawn and/or ppt were detected at all concentrations [78-5000 µg/plate] with TA1535 and TA1537 [except at 78 µg/plate in presence of S9], at concentrations >78 µg/plate with strains TA98, at all concentrations [50-5000 µg/plate] with TA100 [at all but 50 µg/plate in presence of S9], and at concentrations of 500-5000 µg/plate with WP2uvrA.

Definitive study: there were no 2-fold increases in mean revertants with any tester strain, with or without S9. There was, however, a slight but consistent increase in revertants at the highest concentration not associated with a decrease in the background lawn [i.e., 50 µg/plate] with strain TA100 in the presence of S9 [36-53% and 31-36% in Exp 1 and 2, respectively]. Adequate cytotoxicity was obtained with all tester strains, with and without S9.

3. Study title: OPC-31: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells using the

Conducting laboratory and location: _____, Otsuka Study no: 011766, Volume #1.65, Date of study initiation: 1/19/96, GLP, QA'd report: Y]

Drug, lot #, radiolabel, and % purity: OPC-31, lot no. 1H98M. — [CoA provided]

Formulation/vehicle: solution/DMSO; drug concentrations documented to be +/-11% of intended

Methods

Strains/species/cell line: mouse lymphoma L5178Y (*tk*)

Dose selection criteria

Basis of dose selection: cytotoxicity range-finding study

Range finding study: 10-500 µg/mL [+/- S9]

Test agent stability: stability of DMSO solution demonstrated for 6 hrs at ambient temperature

and "scattering light".

Metabolic activation system: male Sprague-Dawley rat liver S9, Δ _____ +-induced

Controls

Vehicle: DMSO

Negative controls: DMSO

Positive controls: 4-nitroquinoline 1-oxide [-S9], benzo(a)pyrene [+S9]

Exposure conditions

Incubation and sampling times: 3 hr treatment [37° C], 2-day expression period, 12-day incubation for determination of TFT resistance

Doses used in definitive study

Exp 1: 8 doses [10-100 µg/mL; -S9], 9 doses [10-100 µg/mL; +S9]

Exp 2: 6 doses [20-60 µg/mL; +/-S9]. It was noted that "...due to slightly low plating efficiencies in the original experiment, the data reported for Experiment 2 are from a repeat experiment".

Analysis

No. of replicates: single for dose-range finding; duplicate (except for PC) for definitive study

Counting method: Coulter counter or hemocytometer [if interference by ppt]

Criteria for positive results: (a) valid assay, (b) MF at one or more concentrations > NC, (c) significant concentration-relationship [linear trend analysis], (d) reproducible results

Comment: colony sizing was performed

Results

Range-finding study: ppt was detected at concentrations of 100-500 µg/mL. OPC-31 was found to be "completely toxic" at concentrations of ≥ 50 µg/mL [-S9] and ≥ 100 µg/mL [+S9]. The HCs with acceptable survival were 20 µg/mL [-S9, RS=134.7%] and 50 µg/mL [+S9, RS=6%].

Definitive studies

Exp 1: ppt was detected after the 3-hr treatment period at the HC [-S9]. RS was <10-20% at the HCs tested, indicating adequate cytotoxicity (and, therefore, concentrations). No increase in MF was noted in the absence of S9. In the presence of S9, there was a significant linear trend for MF; however, there were no significant increases at any one concentration based on analysis using Dunnett's test for multiple comparisons. Increases in MF consistent with positive responses were obtained with both PCs. PCs also produced increases in both large and small colonies.

Exp 2: no ppt was detected. RS was <10-20% at the HC tested, indicating adequate cytotoxicity. There were no increases in MF either with or without S9. PCs produced increases in MF consistent with a positive response. In addition, the PCs produced increases in both small and large colonies.

4. Study title: *In vitro* cytogenetic assay of OPC-14597 using Chinese Hamster Fibroblast Cell Line CHL [Study no: 004525, Volume #1.65, Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceuticals, Ltd., Japan, Date of study initiation: 3/16/88, GLP, QA'd report:]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 870824, purity not stated [CoA not provided]

Formulation/vehicle: solution/DMSO

Methods:

Strains/species/cell line: CHL cells

Dose selection criteria

Basis of dose selection: concentration-finding cytotoxicity study

Range finding study: concentration range: 3.9-500 $\mu\text{g}/\text{mL}$ [8 concentrations, +/-S9]. 6-hr treatment period + 18 hr incubation [+/-S9] and 48-hr treatment period [-S9].

Test agent stability: not documented

Metabolic activation system: rat liver S9 ———— ; no indication of enzyme induction

Controls

Vehicle: DMSO

Negative controls: DMSO

Positive controls: cyclophosphamide [-S9], Mitomycin C [+S9]

Exposure conditions

Incubation and sampling times: 6- and 48-hr treatment [-S9], 6-hr treatment [+S9]. 6-hr treatments were followed by washing and 18 additional hrs of incubation.

Doses used in definitive study

Exp 1: 7.5-60 $\mu\text{g}/\text{mL}$ [4 concentrations, \pm S9, 6-hr treatment], 3.75-60 $\mu\text{g}/\text{mL}$ [5 concentrations, -S9, 48-hr treatment]

Exp 2: 30-60 $\mu\text{g}/\text{mL}$ [3 concentrations, \pm S9, 6-hr treatment]

Exp 3: 15-60 $\mu\text{g}/\text{mL}$ [4 concentrations, -S9, 6-hr treatment], 15-75 $\mu\text{g}/\text{mL}$ [5 concentrations, +S9, 6-hr treatment], 3.75-30 $\mu\text{g}/\text{mL}$ [4 concentrations, -S9, 48-hr treatment]

Analysis

No. of replicates: not clearly stated. 100-200 metaphases were examined per concentration in Exp 1 and 100 metaphases were examined per concentration in Exp 2 and 3.

Method: microscopic evaluation [biomicroscope] of "50 well-spread metaphase cells or more per treatment (one Petri dish)..."

Criteria for positive results: n/s

Results

Range-finding study: the data from the preliminary cytotoxicity test were provided only in a figure. According to the text, 50% growth inhibition was obtained at a concentration of 8 $\mu\text{g}/\text{mL}$ with 48-hr treatment, at 30-45 $\mu\text{g}/\text{mL}$ for the 6-hr treatment without S9, and at 45-60 $\mu\text{g}/\text{mL}$ for the 6-hr treatment with S9.

Exp 1: no cytotoxicity data were provided for this study. Marked increases in the number of cells with structural aberrations [with and without gaps] were observed with and without S9 [6-hr treatment] at the HC [60 $\mu\text{g}/\text{mL}$]. No concentration-related increases in aberrations were observed with the 48-hr treatment [HC = 30 $\mu\text{g}/\text{mL}$]. The data were summarized in the following sponsor's table:

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Table 1 In Vitro Chromosomal Aberration Test of OPC-14597 in Chinese Hamster OHL Cells (Exp.1)

Compound	Concentration	Time (hr)	S9mix (+/-)	Counted Cells	No. of Poly	No. of cells with structural aberrations										Total	
						ctg	ctb	cte	csg	csb	cse	oth	mul	+gap	-gap		
Control (DMSO)	0.5 %			200	0	0	0	0	0	0	0	0	0	0	0	0	0
OPC-14597	7.5 µg/ml	6	-	200	0	1	3	2	0	0	0	0	0	0	6	5	
OPC-14597	15 µg/ml			200	0	1	4	0	0	0	0	0	0	5	4		
OPC-14597	30 µg/ml			200	0	1	1	2	0	4	0	0	0	8**	7**		
OPC-14597	60 µg/ml			toxic													
MPC	0.1 µg/ml			200	0	1	44	60	0	4	1	0	6	83**	83**		
Control (DMSO)	0.5 %			200	0	0	1	1	0	1	0	0	1	3	3		
OPC-14597	7.5 µg/ml	6	+	200	1	1	2	0	0	1	0	0	0	4	3		
OPC-14597	15 µg/ml			200	1	2	2	0	0	1	0	0	0	5	3		
OPC-14597	30 µg/ml			200	3	1	2	0	0	1	0	0	0	3	2		
OPC-14597	60 µg/ml			100	0	0	18	21	0	0	0	2	9	32**	32**		
OPC-14597	60 µg/ml			100	0	1	67	79	0	5	0	0	22	90**	90**		
CP	10 µg/ml			100	0												
Control (DMSO)	0.5 %			200	0	0	0	0	0	0	0	0	0	0	0		
OPC-14597	3.75 µg/ml	48	-	200	0	0	1	1	0	0	0	0	2	2			
OPC-14597	7.5 µg/ml			200	0	0	2	2	0	1	0	0	0	5	5		
OPC-14597	15 µg/ml			100	0	0	3	0	0	0	0	0	0	3	3		
OPC-14597	30 µg/ml			100	0	1	2	0	0	1	0	0	0	4	3		
MPC	0.05 µg/ml			200	0	1	56	95	0	13	0	0	12	125**	125**		

MPC : mitomycin C, CP : cyclophosphamide
 ctg : chromatid gap, ctb : chromatid break, cte : chromatid exchange, csg : chromosome gap, csb : chromosome break, cse : chromosome exchange, oth : other aberrations, mul : multiple aberrations
 +gap : Gaps are included in aberrations
 -gap : Gaps are excluded from aberrations
 Significant differences from control (0.5 % Dimethyl sulfoxide) p<0.01 (***) by Fisher's exact test one-sided

Exp 2: in the absence of S9, relative growth was markedly reduced at 45 and 60 µg/mL [7 and 0% of C, respectively]. The number of cells with structural aberrations was markedly increased at 45 µg/mL. There was no concentration-related increase in cells with structural aberrations in the presence of S9; however, sufficient cytotoxicity [i.e., MI < 50% of C] was not achieved. The data were summarized in the following sponsor's table:

Table 2 In Vitro Chromosomal Aberration Test of OPC-14597 in Chinese Hamster OHL Cells (Exp.2)

Compound	Concentration	Time (hr)	S9mix (+/-)	Counted Cells	No. of Poly	No. of cells with structural aberrations										Total		Relative Growth %
						ctg	ctb	cte	csg	csb	cse	oth	mul	+gap	-gap			
Control (DMSO)	0.5 %			100	0	0	1	2	0	3	0	0	0	5	5	100		
OPC-14597	30 µg/ml	6	-	100	0	0	4	0	0	1	0	0	0	5	5	61		
OPC-14597	45 µg/ml			100	0	0	18	23	0	2	0	0	5	33**	33**	7		
OPC-14597	60 µg/ml			toxic												0		
Control (DMSO)	0.5 %					100	0	0	1	0	0	0	0	0	1	1	100	
OPC-14597	30 µg/ml	6	+	100	0	0	1	3	0	1	0	0	4	4	68			
OPC-14597	45 µg/ml			100	0	0	2	0	0	1	0	0	3	3	71			
OPC-14597	60 µg/ml			100	1	0	2	1	0	1	0	0	4	4	64			

Exp 3: the number of cells with structural aberrations was increased at the HC under all conditions [+/- S9]; the effect with the 48-hr treatment was not statistically significant. Under all 3 conditions, the increase was observed at MI < 50% of C. The data were summarized in the following sponsor's table:

Table 3 *In Vitro* Chromosomal Aberration Test of OPC-14597 in Chinese Hamster CHL Cells (Exp. 3)

Compound	Concentration	Time (hr)	SSmix (+/-)	Counted Cells	No. of Poly	No. of cells with structural aberrations										Total		Relative Growth %
						ctg	ctb	cte	csg	csb	cse	oth	mul	+gap	-gap			
Control (DMSO)	0.5 %			100	0	0	3	2	0	0	0	0	0	0	5	5	100	
OPC-14597	15 µg/ml			100	0	0	3	0	0	1	0	0	0	4	4	112		
OPC-14597	30 µg/ml			100	0	0	3	1	0	2	0	0	0	5	5	95		
OPC-14597	45 µg/ml	6	-	100	0	0	16	23	0	1	0	0	6	30**	30**	3		
OPC-14597	60 µg/ml			toxic														
MPC	0.1 µg/ml			100	0	0	51	40	0	5	0	0	10	64**	64**	100		
Control (DMSO)	0.5 %			100	0	1	2	0	0	2	0	0	0	5	5	100		
OPC-14597	15 µg/ml			100	0	1	2	2	0	1	0	0	0	6	5	82		
OPC-14597	30 µg/ml			100	0	0	2	2	0	1	0	0	1	5	5	47		
OPC-14597	45 µg/ml	6	+	100	0	0	2	1	0	1	0	0	0	4	4	50		
OPC-14597	60 µg/ml			100	0	0	12	13	0	0	0	0	5	20**	20**	27		
OPC-14597	75 µg/ml			toxic														
CP	10 µg/ml			100	0	0	88	96	0	7	0	0	50	100**	100**	100		
Control (DMSO)	0.5 %			100	0	0	2	1	0	1	0	0	0	3	3	100		
OPC-14597	3.75 µg/ml			100	0	0	2	0	0	1	0	0	0	3	3	59		
OPC-14597	7.5 µg/ml			100	0	0	1	0	0	2	0	0	0	3	3	50		
OPC-14597	15 µg/ml	48	-	100	0	0	6	3	0	1	0	0	0	9	9	19		
OPC-14597	30 µg/ml			toxic														
MPC	0.05 µg/ml			100	0	0	74	76	0	12	0	0	38	88**	88**	91		

There appeared to be no effect on polyploidy in any of the experiments.

5. Study title: *In vitro* chromosomal aberration test of OPC-14597 [Study no: 006884, Volume #1.65; Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Japan, Date of study initiation: 1/16/91, GLP, QA'd report: Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 8K89M, [CoA provided]

Formulation/vehicle: solution/DMSO

Methods:

Strains/species/cell line: fibroblasts derived from CHL cells

Dose selection criteria

Basis of dose selection: preliminary cytotoxicity study

Test agent stability: not documented

Metabolic activation system: rat liver S9 [———— Ltd], induced by phenobarbital and 5,6-benzyflavone.

Controls

Vehicle: DMSO

Negative controls: DMSO

Positive controls: cyclophosphamide [6-hr treatment], Mitomycin C [24- and 48-hr treatments]

Exposure conditions

Incubation and sampling times:

Doses used in definitive study:

8.3, 16.5, 33 µg/mL [-S9, 6-hr treatment followed by 18-hr incubation]

13.8, 27.5, 55 µg/mL [+S9, 6-hr treatment followed by 18-hr incubation]

2.5, 5, 10 µg/mL [-S9, 24-hr treatment]

1.3, 2.5, 5 µg/mL [-S9, 48-hr treatment]

Doses used in follow-up study:

20, 30, 40, 50 µg/mL [±S9, 6-hr treatment followed by 18-hr incubation]

Analysis

No. of replicates: duplicates; 100 metaphases were examined per dish = 200 metaphases per concentration.

Counting method: biological microscope

Criteria for positive results: (a) significant increase in incidence of abnormal cells compared to C, (b) concentration-response relationship.

Results

Cytotoxicity study: the data were provided only in graphic form. According to the text, the ID₅₀ [i.e., concentration producing 50% inhibition in cell growth] was 33 µg/mL [-S9] and 55 µg/mL [+S9] with the 6-hr treatment procedure. With the 24- and 48-hr treatments, the ID₅₀ was 10 and 5 µg/mL, respectively. The concentrations selected for the definitive study were equal to, ½, and ¼ of the corresponding ID₅₀.

Definitive study: there was an increase in the no. of cells with structural aberrations at the HC with the 6-hr incubation in the absence of S9. The increases were entirely in chromatid breaks and exchanges. No increases were noted with the 6-hr treatment period in the presence of S9 or with the 24- or 48-hr treatment periods. The data are summarized in the following table:

Table 1 In Vitro Chromosomal Aberration Test of OPC-14597 in Chinese Hamster Cells (CHL)

Compound	Concentration (µg/ml)	Time (hr)	S9mix (+/-)	No. of cells	No. of cells with aberrations								Cells with SA		Poly (X)
					ctg	ctb	cte	csg	csb	cse	oth	+Gap(X)	-Gap(X)		
DMSO	-			200	0	1	0	0	0	0	0	0	0.5	0.5	0.0
OPC-14597	13.8			200	0	1	0	0	1	0	0	1.0	1.0	0.5	
OPC-14597	27.5	8-18	+	200	0	1	1	0	1	0	0	1.5	1.5	4.5 *	
OPC-14597	55			200	0	1	0	0	2	0	0	1.5	1.5	1.5	
CP	10			200	2	10	37	0	0	0	0	23.5 *	23.0 *	0.0	
DMSO	-			200	0	1	1	0	1	1	0	2.0	2.0	0.0	
OPC-14597	8.3			200	1	1	0	0	0	0	1	1.0	0.5	0.0	
OPC-14597	16.5	6-18	-	200	0	0	0	0	0	0	0	0.0	0.0	1.5	
OPC-14597	33			200	0	10	24	0	0	0	0	12.5 **	12.5 **	1.5	
CP	10			200	1	0	0	0	0	0	0	0.5	0.0	0.0	
DMSO	-			200	1	2	0	0	0	0	0	1.5	1.0	0.5	
OPC-14597	2.5			200	1	1	2	0	0	0	0	2.0	1.5	0.5	
OPC-14597	5.0	24-0	-	200	0	0	0	0	0	0	0	0.0	0.0	0.0	
OPC-14597	10			200	0	1	1	0	2	0	0	2.0	2.0	0.0	
MWC	0.04			200	1	47	59	0	2	2	0	43.0 *	43.0 *	0.5	
DMSO	-			200	0	0	0	0	0	2	0	1.0	1.0	0.0	
OPC-14597	1.3			200	1	2	1	0	0	1	0	2.5	2.0	0.0	
OPC-14597	2.5	48-0	-	200	0	0	1	0	2	0	0	1.5	1.5	0.0	
OPC-14597	5			200	0	1	1	0	0	0	0	0.5	0.5	0.5	
MWC	0.04			200	1	108	127	1	5	5	1	72.5 *	72.5 *	0.5	

CP : cyclophosphamide, MWC : mitomycin C, Time : treatment-recovery, ctg : chromatid gap, ctb : chromatid break, cte : chromatid exchange, csg : chromosome gap, csb : chromosome break, cse : chromosome exchange, oth : other aberration, SA : structural aberrations, +Gap : Gaps are included in aberrations, -Gap : Gaps are excluded from aberrations, Poly : polyploid. Significant differences from control (DMSO, 0.5X) at p<0.01 (+) by Fisher's exact test one-sided. Significantly dose-related increase in the frequency at p<0.01 (#) by Cochran-Armitage trend test one-sided.

No cytotoxicity data were provided for this assay. The PCs produced responses consistent with a positive response under all conditions used.

Follow-up study: there was an increase in the no. of cells with structural aberrations at the HC tested with the 6-hr treatment period in the absence of S9; no increase was noted in the presence of S9. Adequate cytotoxicity was not obtained in the presence of S9 [relative survival was 78% at the HC]; in the absence of S9, relative survival was 35% at the HC [concentration associated with the positive response]. The data were provided in the following table:

Table 2 *In Vitro* Chromosomal Aberration Test of OPC-14597 in CHL Cells (Repeat Test)

Compound	Concentration ($\mu\text{g}/\text{ml}$)	Time (hr)	S9mix (+/-)	No. of cells	No. of cells with aberrations								Cells with SA		Poly (%)	Survival (%)
					ctg	ctb	cte	cag	csb	cse	oth	+Gap(X)	-Gap(X)			
DMSO	-			200	0	1	0	0	0	0	0	0	0.5	0.5	1.0	100
OPC-14597	20			200	0	1	2	0	1	0	0	2.0	2.0	2.5	88	
OPC-14597	30	6-18	+	200	0	0	1	0	0	0	2	0.5	0.5	1.0	93	
OPC-14597	40			200	0	1	2	0	0	0	0	1.0	1.0	2.0	97	
OPC-14597	50			200	0	1	3	0	0	0	0	1.5	1.5	2.0	78	
CP	10			200	0	55	113	0	3	4	0	63.5 *	63.5 *	0.0	61	
DMSO	-			200	0	0	0	0	0	0	1	0.0	0.0	1.0	100	
OPC-14597	20			200	1	1	1	0	0	0	0	1.5	1.0	2.5	78	
OPC-14597	30	6-18	-	200	0	6	18	0	0	2	2	11.0 **	11.0 **	1.5	35	
OPC-14597	40			NT	-	-	-	-	-	-	-	-	-	-	1	
OPC-14597	50			NT	-	-	-	-	-	-	-	-	-	-	2	
CP	10			200	0	0	0	0	1	1	0	1.0	1.0	0.0	95	

6. Study title: *In vitro* chromosomal aberration test of OPC-14597 (Supplement 1) [Study no: 009719, Volume #1.65, Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Japan, Date of study initiation: 12/9/93, GLP, QA'd report: Y]

Purpose: this study was a supplement to Study No. 006884 [#5 in this section].

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 93H80M1 — [CoA provided]

Formulation/vehicle: solution/DMSO. Solutions were stated to have been stable for 6 hrs at rm temperature and "scattered light"; no supportive data were provided.

Methods:

Strains/species/cell line: fibroblast cell line from CHL

Dose selection criteria

Basis of dose selection: data from previous study [Study No. 006884]

Range finding studies: none

Test agent stability: Solutions were stated to have been stable for 6 hrs at rm temperature and "scattered light"; no supportive data were provided

Metabolic activation system: rat liver S9 [phenobarbital and 5,6-benzoflavone-induced] —

Controls

Vehicle: DMSO

Negative controls: DMSO

Positive controls: Mitomycin C [-S9], cyclophosphamide [+S9]

Exposure conditions:

Incubation and sampling times: 6-hr treatment, followed by washing, and 18 hrs culture time.

Doses used in definitive study: 10, 20, 30, 40 $\mu\text{g}/\text{mL}$ [-S9], 30, 40, 50, 60 $\mu\text{g}/\text{mL}$ [+S9]

Analysis:

No. of replicates: 4 per treatment grp [2 for analysis of chromosomal aberrations, 2 for cell counting]

Counting method: 100 metaphases were analyzed per treatment dish, or 200 metaphases per

concentration, for chromosomal aberrations. Cell counting was performed using a biomicroscope _____]

Criteria for positive results: (a) a significant increase in the incidence of abnormal cells and (b) positive trend test.

Results

OPC-14597 produced significant and concentration-related increases in the no. of cells with chromosomal aberrations in the absence and presence of S9. The data were summarized in the following sponsor's table:

Table 1 The cytogenetic effect of OPC-14597 on cultured CHL cells

Compound	Concentration (µg/mL)	Time (hr)	S9mix (+/-)	No. of cells analyzed	Numbers of cells with structural aberrations							Frequencies (%) of cells with SA		Frequencies (%) of cells with Other Poly	
					ctg	ctb	cte	cag	csb	cse	mul	+Gap	-Gap	Other	Poly
DMSO (control)	(0.5%)			200	0	5	1	0	3	0	1	4.0	4.0	1.0	0.0
OPC-14597	10	6-18	-	200	1	3	1	0	0	0	0	2.5 [#]	2.0 [#]	0.5	2.5 [#]
OPC-14597	20			2	1	1	0	0	0	0	2.0 [#]	1.0 [#]	0.0	2.0 [#]	
OPC-14597	30			3	8	15	0	0	1	3	10.5 ^{#*}	10.0 ^{#*}	0.5	0.5 [#]	
OPC-14597	40			3	75	49	0	1	0	45	70.2 ^{#*}	70.2 ^{#*}	0.0	0.8 [#]	
Mitomycin C	0.1			200	2	86	74	1	14	0	12	65.5 [*]	65.5 [*]	0.5	1.0 [#]
DMSO (control)	(0.5%)			200	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0
OPC-14597	30	6-18	+	200	0	1	0	0	0	1	0	1.0 [#]	1.0 [#]	0.0	7.0 [*]
OPC-14597	40			0	1	1	0	0	0	0	0.5 [#]	0.5 [#]	0.5	5.0 [*]	
OPC-14597	50			0	1	0	0	0	1	0	1.0 [#]	1.0 [#]	0.0	2.5 [#]	
OPC-14597	60			0	8	13	0	0	0	0	8.0 ^{#*}	8.0 ^{#*}	0.0	0.5 [#]	
Cyclo-phosphamide	10			200	0	23	44	0	10	1	0	33.5 [*]	33.5 [*]	0.0	0.5 [#]

Time :treatment-recovery, SA :structural aberrations, ctg :chromatid gap, ctb :chromatid break, cte :chromatid exchange, cag : chromosome gap, csb :chromosome break, cse :chromosome exchange, mul :multiple aberration ;more than 5 aberrations, +Gap :gaps are included in aberrations, -Gap :gaps are excluded from aberrations, Other :other aberrations except structural aberrations, Poly :polyploidy.

Significant differences from solvent control at P<0.05(*) or not(N) by Fisher's exact test one-sided.

Significantly dose-related increase in the frequency at P<0.01(#) by Cochran-Armitage trend test one-sided.

The sponsor noted an increase in the number of structural aberrations per cell at 30 and 40 µg/mL in the absence of S9 [42/200 and 327/124 cells, respectively, vs 14/200 cells for negative C] and at 60 µg/mL in the presence of S9 [25/200 cells, respectively, vs 0/200 cells for negative C]. Cytotoxicity data were not provided. It was stated in the report that relative survival was 100, 94, 77, and 20% at 10, 20, 30, and 40 µg/mL in the absence of S9; cells could not be accurately counted in the presence of S9 "...due to residue of S-9".

The number of cells with polyploidy was increased; however, there was a negative trend both in the absence and presence of metabolic activation.

7. Study title: Preliminary micronucleus test of OPC-14597 in bone marrow erythrocytes after a single oral administration of OPC-14597 to ICR mice [Study no: 005784, Volume #1.65, Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceuticals Co., Ltd., Japan, Date of study initiation: 8/4/89, non-GLP, QA'd report: N]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 8K84M, _____ (CoA provided)

Formulation/vehicle: suspension/5% gum arabic [5-15 ng/mL

Methods:

Strains/species/cell line: ICR mouse _____], n = 6/sex/grp for treatment grps sacrificed on Days 0 and 1; n = 4/sex/grp for treatment grps sacrificed on Day 2; n = 2/sex/grp for treatment grps sacrificed on Day 3. N for NCs was 3/sex for Days 0 and 1, 2/sex for Day 2, and 1/sex for Day 3 sacrifice.

Dose selection criteria

Basis of dose selection: preliminary dose-range finding study.

Range finding study: deaths occurred at 93 mg/kg [1/3 F] and at ≥ 327 mg/kg [all males and females].

Test agent stability: stated to have been stable in suspension for 11 days and stored at room temperature protected from light [CoA not provided].

Metabolic activation system: n/a

Doses used: 0, 50, 75, 100, 150 mg/kg

Observations:

Clinical signs: animals were observed twice per day until sacrifice.

Body wt: animals were weighed prior to dosing and daily until sacrificed.

Terminal sacrifice: animals were sacrificed on Day 1, 2, and 3 [dosing: Day 0]

Bone marrow collection: samples collected from femur. Prepared cells were stained with acridine orange immediately prior to evaluation using an epifluorescent microscope.

Analysis: cytotoxicity was determined based on examination of 1000 erythrocytes/animal; micronuclei were assessed on 2000 PCE/animal.

Criteria for positive results: n/s

Results

Mortality: there was one unscheduled death [1 HDF, Day 1, 19 hrs postdosing].

Clinical signs: reduced SMA, crouching position, and ptosis were observed at all doses, in all animals. At the HD, tonic convulsions were observed in 1 M at ≈ 19 hrs postdosing.

Body wt: there was a dose-related decrease in body wt in males [Day 3: 7 and 11% at 100 and 150 mg/kg, respectively] compared to CM. Body wt tended to be lower in dosed groups in females; however, the differences were not consistently dose-related.

Analysis: due to the death and convulsions observed at the HD, the HD was considered to have exceeded the MTD and the data were removed from the overall analysis. Individual data were not provided.

No bone marrow cytotoxicity was evident. The micronuclei data were summarized in the following tables:

Table-2 The frequency of micronucleated polychromatic erythrocytes (MNPCEs) after the single peroral administration of OPC-14597 to ICR mice

SEX: Male

DAY	DOSE (mg/kg)					Row total / n %	150
	0	50	75	100			
1	6	6 2	5 4	5 8	30/6 2.5 \pm 1.0	7 5	
2	4	3 5	5 1	6 6	26/6 2.2 \pm 1.0	7 7	
--3	4	4 3	3 4	6 6	26/6 2.2 \pm 0.7	(13) 3	
Column total / n %		23/6 1.9 \pm 0.7	22/6 1.8 \pm 0.8	37/6 3.1 \pm 0.5			

SEX: Female

DAY -	DOSE (mg/kg)					
	0	50	75	100	Row total / n %	150
1	6	5 5	3 3	4 3	23/6 1.9±0.5	7 nd
2	3	3 6	9 7	11 9	45/6 3.8±1.4	15 12
3	14	6 5	9 7	4 7	38/6 3.2±0.9	16 3
Column total / n %		30/6 2.5±0.5	38/6 3.2±1.4	38/6 3.2±1.6		

It is unclear what the 2 columns of data [within each dose grp] represent. For example, there was a total of 6/sex/grp for the Day 1 analyses, not 2/sex/grp. The column and row totals are not helpful since they combine data that should not be combined.

8. Study title: Micronucleus test of OPC-14597 in bone marrow erythrocytes after a single oral administration to ICR mice [Study no: 005785, Volume #1.65, Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Japan, Date of study initiation:9/19/89, GLP, QA'd report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 8K89M, ——— [CoA provided]

Formulation/vehicle: suspension/5% gum arabic

Methods:

Strains/species/cell line: ICR mouse ! ———, 5/sex/grp

Dose selection criteria

Basis of dose selection: range-finding study

Range finding study: previously reviewed [#7, this section]

Test agent stability: stated to be stable "up to the end of the experiment"; no CoA or other supportive data provided.

Metabolic activation system: n/a

Controls:

Vehicle: 5% gum arabic

Negative controls: vehicle

Positive controls: Mitomycin C [5 mg/kg]

Exposure conditions

Incubation and sampling times: animals were sacrificed 2 days [≈48 hrs] following a single dose.

Doses used in definitive study: 0, 25, 50, 100 mg/kg p.o.

Analysis

Methods:

No. of replicates: the number of PCEs and NCEs were based on examination of 1000 erythrocytes per animal; micronuclei were counted in 2000 PCEs per animal.

Counting method: microscopic evaluation.

Criteria for positive results: n/s

Results

There were no unscheduled deaths during the study. Drug-related clinical signs, consisting of reduced spontaneous motor activity and ptosis, were observed in all animals at all doses. There was no mention

of "cold to touch" or hypothermia in any animal. The duration of the clinical signs was longer at the higher doses. Mean body wt was reduced at all doses in males on Day 1 [8, 13, and 10% at LD, MD, and HD, respectively] and Day 2 [9 and 12% at MD and HD, respectively] postdosing, and in HDF on Days 1 and 2 [7%].

There was no evidence of bone marrow cytotoxicity [PCE:total erythrocytes was similar among grps]. The frequency of micronucleated PCEs [%MN-PCE] was slightly higher at the HD in both males and females compared to Cs; however, the differences were not statistically significant. The data were summarized in the following sponsor's table:

Table 2 The frequencies of micronucleated polychromatic erythrocytes (PCEs) and PCE ratios among erythrocytes after a single peroral administration of OPC-14597 to ICR mice

Sex	Micronucleus frequency 1)		PCE ratio 2)	
	Mean ± S.D.	Range	Mean ± S.D.	Range
Male				
0	2.5 ± 1.6	1.0 ~ 5.0	56 ± 8	
25	2.1 ± 0.8	1.5 ~ 3.0	55 ± 9	
50	2.3 ± 1.0	1.0 ~ 3.0	52 ± 14	
100	3.5 ± 2.0	1.0 ~ 6.5	54 ± 14	
MMC5	16.1 ± 11.1*	6.0 ~ 28.0	53 ± 3	
Female				
0	2.6 ± 0.8	1.5 ~ 3.5	55 ± 13	
25	2.6 ± 1.1	1.5 ~ 4.5	58 ± 14	
50	2.8 ± 0.9	2.0 ~ 4.0	55 ± 5	
100	4.4 ± 2.6	0.0 ~ 6.5	54 ± 7	
MMC5	16.0 ± 5.3*	10.0 ~ 20.0	49 ± 8	

1) The permillage of micronucleated PCE in 2000 PCEs per body
 2) The percentage of PCE in 1000 erythrocytes per body
 S.D.: Standard deviation, MMC: Mitomycin C
 * : Significant difference from the control (p < 0.01)

Individual animal data indicated that %MN-PCE ranged from 2-10/2000 in CM and 2-13/2000 in HDM, and from 3-7/2000 in CF and 0-13/2000 in HDF. In HDF, 1F had a %MN-PCE of 0/2000; if that value were omitted, the %MN-PCE for the HDF would be 11/2000 or ≈5.5%.

9. Study title: Preliminary micronucleus test of OPC-14597 in bone marrow erythrocytes after a single oral administration to ICR mice-II-[Study no: 014702, Volume #1.66, Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Date of study initiation: 5/26/98, GLP, QA'd report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 97A99M, — [CoA provided]

Formulation/vehicle: suspension/5% gum arabic

Methods:

Strains/species/cell line: CD-1 [ICR] mouse [—], 3/sex/grp per time point.

Dose selection criteria

Basis of dose selection: preliminary single-dose toxicity study, previous *in vivo* micronucleus

assay.

Range finding studies: death observed in all animals at 327 mg/kg p.o., and in 1/3 F at 93 mg/kg in the acute toxicity study. In the *in vivo* micronucleus assay, deaths occurred in F and tonic convulsions were observed in 1 M at 150 mg/kg.

Test agent stability: stated to be stable in suspension for "11 days in a cool place..."; no CoA or other data provided to document stability.

Metabolic activation system: n/a

Controls

Negative controls: vehicle

Positive controls: Mitomycin C

Exposure conditions

Incubation and sampling times: animals were sacrificed at 24 and 48 hrs postdosing

Doses used: 0, 75, 100, 150, 200, and 300 mg/kg p.o. [single dose]; the 300-mg/kg dose was added after no deaths occurred at 200 mg/kg.

Observations:

Clinical signs: animals observed at 30 min, 1, 2, and 3 [HD only] hrs postdosing on Day 0, twice on Day 1 and 2, and during body wt recording.

Body wt: recorded prior to dosing, at 24 hrs postdosing, and prior to sacrifice.

Gross pathology: animals were necropsied.

Analyses:

No. of replicates: the PCE and NCE ratios were determined based on examination of 1000 erythrocytes per animal; the number of MNPCs was based on examination of 2000 PCEs per animal.

Counting method: bone marrow preparations were stained with acridine orange immediately prior to examination with epifluorescence microscope.

Criteria for positive results: n/s

Results

Two of 6 HDF died prematurely. HDF 00074 was found dead; HDF 00076 died immediately following a convulsion on Day 1. [Due to these deaths, %MN-PCE was not determined in HDF; %MN-PCE were also not assessed at the LD.] Prior to death, animals exhibited reduced SMA, prone posture, ptosis, and convulsions [Day 0]. HDF survivors exhibited reduced SMA, prone posture, and ptosis on Days 0 and 1. Similar clinical signs were evident at the lower doses. There were no unscheduled deaths in males. Clinical signs were evident at all doses [including reduced SMA, prone posture, crouching position, and/or ptosis]. Body wt tended to be lower in dosed grps; however, the effect was not clearly dose-related in either males or females. There were no drug-related gross lesions.

No dose-related cytotoxicity was observed at the 24-hr sampling time. At the 48-hr sampling time, the %PCEs was reduced at 200 and 300 mg/kg in males [17 and 32%, respectively] and 200 mg/kg [19%] in females. %MN-PCE was not increased in either males or females at the 24-hr sampling time. At the 48-hr sampling time, %MN-PCE was slightly increased at 200 mg/kg in males [36/6000 vs 16/6000 in CM] and in females [29/6000 vs 18/6000 in CF]. The PC was tested only at the 24-hr sampling time; %MN-PCE was significantly increased in both males and females.

10. Study title: Supplementary test in male to preliminary micronucleus test of OPC-14597 in bone marrow erythrocytes after a single oral administration to ICR mice-II-[Study no: 014801, Volume #1.66, Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Date of study initiation:8/4/98, GLP, QA'd report:Y]

Purpose: to test male mice at higher doses since no lethality was observed in males in previous *in vivo* micronuclei assays.

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 97A99M. — [CoA provided]

Formulation/vehicle: suspension/5% gum arabic

Methods:

Strains/species/cell line: male CD-1 [ICR] mouse 1 —————, 3/grp

Dose selection criteria

Basis of dose selection: previous studies

Range finding studies: Y

Test agent stability: stated to have been confirmed by re-analysis after use; however, no documentation was provided.

Metabolic activation system: n/a

Controls

Negative controls: none

Positive controls: none

Exposure conditions

Doses used in study: 500, 1000, 2000 mg/kg p.o. [single dose]

Observations

clinical signs: all animals were observed at 30 min, 1, 2, and 3 hrs postdosing, twice daily on Days 1 and 2, and when weighed.

body wts: recorded prior to dosing, at 24 hrs postdosing, and prior to sacrifice on Day 2.

terminal: all animals were sacrificed 48 hrs postdosing, and thoracic and abdominal internal organs were examined macroscopically.

analysis

No. of replicates: bone marrow samples were collected from LD survivors.

Samples were prepared in duplicate. 1000 erythrocytes per animal were examined for cytotoxicity [i.e., PCE:total erythrocyte ratio]; 2000 PCEs were examined per animal for determination of %MN-PCE.

Counting method: samples were stained with acridine orange immediately prior to examination with an epifluorescence microscope.

Criteria for positive results: n/s

Results

Unscheduled deaths occurred at 1000 mg/kg [1/3 found dead, Day 1] and 2000 mg/kg [2/3 found dead, Day 1-2]. Clinical signs in these animals consisted of decreased SMA, prone position, ptosis, convulsions [1 HD]. In all survivors, reduced SMA, ptosis, prone position, coldness to touch, tremor, and convulsions were also observed. Body wt decreased during the observation period at the LD and MD. %MN-PCE was determined only at the LD. The %PCE and %MN-PCE were 44 ± 7 and $15/6000$ [mean: 2.5 ± 1.3 % MNPCEs], respectively.

11. Study title: Micronucleus test of OPC-14597 in bone marrow erythrocytes after a single oral administration to ICR mice-II-[Study no: 014327, Volume #1.66, Conducting laboratory and location: Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Date of study initiation: 9/18/98, GLP, QA'd report: Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 98A91M, — CoA provided]

Formulation/vehicle: suspension/5% gum arabic

Methods

Strains/species/cell line: CD-1 [ICR] mouse 1 —————, 5/sex/grp [3/sex/grp for PC] per sacrifice time [24, 48-hr]

Dose selection criteria

Basis of dose selection: previous studies

Range finding studies: Y

Test agent stability: stated to have been demonstrated previously; no documentation provided.

Metabolic activation system: n/a

Controls

Negative controls: vehicle

Positive controls: Mitomycin C

Exposure conditions

Doses used in definitive study: 0, 50 [F only], 100, 200, 500 [M only] mg/kg [single oral dose]

Observations

Clinical signs: animals were observed at 30 min, 1, 2, 3, 18, 23, 42, and 47 hrs postdosing. Ten/sex/grp were observed on Day 0 and Day 1, and 5/sex/grp were observed on Day 2.

Body wt: recorded prior to dosing, on Day 1, and prior to sacrifice [Day 1 or 2]

TK: blood samples were collected from all animals [except 1 HDM that died] at 24 and 48 hrs postdosing [at time of bone marrow sampling] for analysis of drug levels [reported separately].

Terminal: animals were sacrificed either 24 or 48 hrs postdosing. Thoracic and abdominal organs were examined for gross lesions.

Bone marrow analysis

No. of replicates: bone marrow samples were collected [femur] from all grps except the HD [due to deaths]. Bone marrow smears were prepared in duplicate for each animal. Samples were stained with acridine orange just prior to examination.

Counting method: epifluorescence microscopy. The PCE:NCE ratio was determined on 1000 erythrocytes per animal; the number of MN was determined in 2000 PCEs per animal. MN were classified as large or small [i.e., < or > 1 μ m].

Criteria for positive results: (a) %MN-PCE in any one dose grp > upper limit [mean + 2 SD of 4.3% for males, 4.8% for females] based on HC data. (b) %MN-PCE increased in a dose-dependent manner, and/or (c) %MN-PCE significantly greater in any treatment grp compared to NC.

Results

There was 1 unscheduled death; 1 HDM was found dead on Day 1. [Due to the death in this M, %MN-PCEs were not assessed at this dose.] Clinical signs observed in this animal consisted of decreased SMA, prone posture, ptosis, cold to touch, tremors, and convulsion [Day 0]. Convulsions were also observed in male survivors at 200 and 500 mg/kg. Convulsions were also observed in 4/10 F at 200 mg/kg. Drug-related clinical signs [decreased SMA, prone position, coldness to touch, ptosis] were evident at all doses. Body wt was reduced in males at all dose; however, the effect was not dose-related [Day 1: 6, 5, and 3% at LD, MD, and HD, respectively; Day 2: 12, 11, and 7% at LD, MD, and HD, respectively]. In females, body wt was also reduced at all doses; the effect was transient at the LD [Day 1: 8%] but consistent at the MD [6-8%] and HD [4-9%].

At the 24-hr sampling time, the %PCEs was similar among grps in both males and females, i.e., there was no evidence of cytotoxicity. There was also no difference in %MN-PCE among grps. At the 48-hr sampling time, the %PCEs was fairly similar among grps, i.e., no evidence of cytotoxicity. %MN-PCE was significantly increased at both doses in males and increased in females at all doses [increases were statistically significant at the MD and HD]. The 48-hr data were summarized in the following sponsor's table:

Table 3-3
Micronucleus Test of OPC-14597 in Bone Marrow Erythrocytes after a Single Oral Administration to ICR Mice -11-
Item : Bone Marrow - Micronucleus Count: 48-hour sampling
SEX : Male **Statistical control : A0**

Dose		A0	A100	A200
XPOE	[MEAN, SD]	52±6	57±8	58±12
	[MIN-MAX]	5-10	5-15	5-20
XMNPC	[MNPC/PCE]	16/100000#	32/10000*	37/10000**
	[n]	5	5	5
XMPCE	[MEAN, SD]	1.6± 0.4	3.2± 1.8	3.7± 0.8
	[MIN-MAX]	1.0- 2.0	1.0- 5.0	3.0- 4.5
	[n]	5	5	5

A0 : 5 % Acacia A50 : OPC-14597 50 mg/kg A100 : OPC-14597 100 mg/kg
 * ; P<0.05 ; ** ; P<0.01 ; Significant difference from the control (one tailed)
 ## ; P<0.01 ; Significant dose dependency (one tailed)

Table 3-4
Micronucleus Test of OPC-14597 in Bone Marrow Erythrocytes after a Single Oral Administration to ICR Mice -11-
Item : Bone Marrow - Micronucleus Count : 48-hour sampling
SEX : Female **Statistical control : A0**

Dose		A0	A50	A100	A200
XPOE	[MEAN, SD]	52±7	55±11	51±8	52±6
	[MIN-MAX]	5-10	5-15	5-15	5-15
XMNPC	[MNPC/PCE]	18/100000#	25/10000	35/10000*	45/10000**
	[n]	5	5	5	5
XMPCE	[MEAN, SD]	1.8± 1.0	2.5± 0.8	3.5± 1.5	4.5± 1.8
	[MIN-MAX]	0.5- 3.0	1.5- 3.5	1.5- 5.5	2.5- 6.0
	[n]	5	5	5	5

A0 : 5 % Acacia A50 : OPC-14597 50 mg/kg A100 : OPC-14597 100 mg/kg
 A200 : OPC-14597 200 mg/kg
 * ; P<0.05 ; ** ; P<0.01 ; Significant difference from the control (one tailed)
 ## ; P<0.01 ; Significant dose dependency (one tailed)

The sponsor noted that "a few" individual values at 100 and 200 mg/kg [males and females] exceeded the high-C values. An examination of the individual data indicated that values exceeded the high-C value [48-hr data] in 3/5 and 5/5 males at 100 and 200 mg/kg, respectively, and in 1/5, 3/5, and 4/5 females at 50, 100, and 200 mg/kg, respectively.

The sponsor attributed the positive responses with aripiprazole to hypothermia. Body temperature was not measured in this study; however, the sponsor cited the results from Study No. 015132 as evidence that aripiprazole produces hypothermia at doses of 50 ["lowest mean temperature" = 29° C], 100 mg/kg and 200 mg/kg ["lowest mean temperature" = 24° C]. The sponsor also noted that the decrease in body temperature lasted from 2 to 24-48 hrs at doses of 100 and 200 mg/kg, and from 2 to 19 hrs postdosing at 50 mg/kg.

The sponsor considered aripiprazole to be negative in this test.

12. Study title: *In vivo-in vitro* hepatocyte DNA repair assay of OPC-31 in male F-344 rats [Study no: 009961, Volume #1.65, Conducting laboratory and location: _____, Date of study initiation: 4/21/94, GLP, QA'd report:]

Drug, lot #, radiolabel, and % purity: OPC-31, lot no. 93H80M1 _____ [CoA not provided]

Formulation/vehicle: suspension/5% gum arabic

Methods:

Strains/species/cell line: male Fischer 344 rat _____ [4/grp/time point]

Dose selection criteria

Basis of dose selection: dose-range finding study

Range finding study: conducted in male F-344 rats [3/grp] at doses of 125-2000 mg/kg p.o. Doses of 500-2000 mg/kg were associated with severe clinical signs [e.g., ataxia, prostration, hypothermia, tremors, labored breathing] and animals were sacrificed moribund on Day 3 postdosing.

Test agent stability: stated to have been documented; CoA was not provided. Analysis of dosing suspensions [2 and 16-hr] indicated that achieved concentrations were 99-105% of intended.

Metabolic activation system: n/a

Controls

Negative controls: vehicle

Positive controls: dimethylnitrosamine

Exposure conditions:

Incubation and sampling times: animals were sacrificed 2 and 16 hrs postdosing.

Doses used in definitive study: 0, 125, 250, 500 mg/kg

Study design: 2 experiments were conducted. In the 1st experiment, the dosing suspension was determined not to be stable [i.e., suspension formed a cake-like formation at the bottom of the container which could not be re-suspended], resulting in inadequate dosing for the 2-hr sampling time. Therefore, a 2nd experiment was conducted to re-assess the 2-hr time point.

Analysis:

No. of replicates: "whenever possible", 3 slides were scored per animal.

Counting method: slides were examined using light microscopy [quantitative autoradiographic grain counting. "At least 30 morphologically unaltered cells..." were counted per slide; therefore, ~90 cells were examined per animal.

Criteria for positive results: the report defined "unequivocally positive" results as a mean net grain count >0 and the % cells in repair >20% for any dose grp.

Results

Clinical signs in the definitive studies were not discussed nor were data provided. There were no increases in the % cells in repair at any dose of OPC-31. Only one set of data was provided for the 2-hr time point; it is assumed that this was the dataset for the repeat 2-hr assay. The positive control produced increases in % cells in repair consistent with a positive response at both sampling times. Data for individual slides were not provided

TK Data

TK data were provided for the *in vivo* genotoxicity studies as indicated below:

1. Study title: **Plasma concentrations of OPC-14597 in ICR mice after single oral administration at 100 mg/kg** [Study no: 009379; data for *in vivo* chromosomal aberration assay, Study No. 005932].
2. Study title: **Plasma concentration assay of OPC-14597 in ICR mice after a single oral administration-II**-[Study no: 014978; data for *in vivo* chromosomal aberration assay, Study No. 014327].
3. Study title: **Plasma concentrations assay of OPC-14597 and its metabolites after a single oral administration at 200 mg/kg in ICR mice** [Study no: 015644; data for *in vivo* chromosomal aberration assay, Study No. 013049].
4. Study title: **Plasma concentrations of OPC-14597 in male F344 rats after a single oral administration** [Study no: 009379; data for *in vitro-in vivo* unscheduled DNA synthesis assay, Study No. 008787].

The data from these reports are summarized in the following table:

STUDY	COMPOUND	DOSE (mg/kg)	MALES		FEMALES	
			C _{max} [ng/mL]	AUC [ng•hr/mL]*	C _{max} [ng/mL]	AUC [ng•hr/mL]
009379	OPC-14597	100	3070		3150	
014978		200	4981	89499 ± 127532	4180	82496 ± 118350
015644		200	3410	89258	4153	94646
014322		500	6584	70827		
015644	OPC-14857	200	750	26581	1005	31000
	DM-1451		6	178	14	277
	DM-1452		580	15913	684	16733
	DCPP		147	4291	123	3568
	OPC-3373		336	6186	1044	19251

* Study 015644, 014978: 0-48 hrs; Study 014322: 0-16 hr postdosing

Metabolites

The genotoxic potential of SFO-14018 [1.22-5000 µg/plate], a synthetic intermediate of OPC-14597, was tested in an Ames assay using *Salmonella typhimurium* tester strains, TA1535, TA1537, TA100, and TA98, and *E. coli* strain WP2uvrA. Testing was conducted in the absence and presence of metabolic activation [male Sprague-Dawley rat liver S9]. The following positive controls were used: AF-2 [TA100, TA98, WP2uvrA], NaN₃ [TA1535], ACR [TA1537] in the absence of S9, and 2-AA for all tester strains in the presence of S9. Tests were conducted in triplicate in two separate experiments. No increases in revertants were detected without or with S9 for any tester strain.

Genetic Toxicology Summary and Conclusions

The genotoxic potential of aripiprazole was assessed in the following studies: *in vitro* bacterial DNA repair [*Bacillus subtilis*], *in vitro* bacterial reverse mutation [Ames] assay [*S. typhimurium* strains, TA1535, TA1537, TA100, TA98, *E. coli* WP2uvrA], *in vitro* mouse lymphoma [with colony sizing], *in vitro* cytogenetics assay in CHL cells, *in vitro* chromosomal aberration assay in fibroblasts derived from CHL cells [2 studies], *in vivo* micronucleus assay in ICR mice [2 studies], *in vivo-in vitro* hepatocyte DNA repair assay in male F-344 Fischer rats.

Aripiprazole was negative in the *in vitro* bacterial DNA repair, the Ames, and the *in vitro* mouse lymphoma assays. In the mouse lymphoma assay, a positive linear trend was obtained in the presence of rat liver S9. However, mutation frequency [MF] was not significantly increased at any concentration, and no increases in MF were observed in a repeat assay.

Aripiprazole produced significant, reproducible increases in structural chromosomal aberrations, both in the absence and presence of metabolic activation [rat S9], in a series of *in vitro* chromosomal aberration assays in fibroblast derived from Chinese hamster lung [CHL]. The 6-hr treatment MF [- gaps] data from these studies are summarized in the table below. The numbers in parentheses indicate % relative growth/survival. The unavailability of cytotoxicity data for all experiments made it difficult to completely assess the correlation between positive responses and cytotoxicity. However, it is clear that positive responses were obtained both without and with S9 in the absence of severe cytotoxicity. [In two experiments in which no increases in MF were obtained in the presence of S9, high concentrations should have been used, as evidenced by a lack of sufficient cytotoxicity.] It should be noted that it is not generally acknowledged that positive responses in this assay should be ignored if associated with

excessive cytotoxicity [i.e., RS/RG <10-20%]. Therefore, these data suggest that aripiprazole has both direct and indirect clastogenic potential.

CONC [µg/mL]	STUDY 004525						STUDY 006884				STUDY 009719	
	Exp [#] 1		Exp 2		Exp 3		Exp [#] 1		Ex 2		-S9	+S9 [#]
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9		
0	0	3	5	1	5	5	2.0	0.5	0.0	0.5	4.0	0
7.5	5	3										
8.3							0.5					
10											2.0	
13.8								1.0				
15	4	3			4	5						
16.5							0.0					
20									1.0	2.0	1.0	
27.5								1.5				
30	7 ^{**}	2	5 [61]	4	5	5			11.0 ^{**} [35]	0.5	10.0 [*] [77]	1.0
33							12.5 ^{**}					
40										1.0	70.2 ^{**} [20]	0.5
45			33 ^{**} [7]	3	30 ^{**} [3]	4						
50										1.5 [78]		1.0
55								1.5				
60	toxic	32 ^{**}	toxic	4 [64]	toxic	20 ^{**} [27]						9.0 ^{**}
75						toxic						

^{*}p<0.05, ^{**}p<0.01, [#]RG/RS data not provided

Two definitive *in vivo* micronucleus assays were conducted. In preliminary studies conducted using acute doses of 50-300 mg/kg p.o., doses of 150 and 300 mg/kg in females and of 1000 and 2000 mg/kg in males exceeded the MTD based on spontaneous deaths occurring at those doses. Doses used in the definitive studies were 0, 25, 50, and 100 mg/kg and 0, 50 (F only), 100, 200, and 500 [M only] mg/kg p.o. One M dosed at 500 mg/kg died spontaneous; no deaths occurred at the lower doses in either study. In the lower-dose range study [Study 005798], the % micronucleated PCEs [%MN-PCE] was determined only at 48 hrs postdosing; both 24- and 48-hr sampling times were examined in the higher-dose range study [Study 014327]. There was no evidence of bone marrow cytotoxicity in either study. [A decrease in %PCEs, evidence of bone marrow cytotoxicity, was, however, noted in a preliminary study at doses of 200 and 300 mg/kg in males (17-32%) and at 200 mg/kg in females (19%)]. There was no significant increase in %MN-PCE in Study 005798, although the %MN-PCE tended to be higher at the HC in both males and females. In Study 014327, the %MN-PCE was not affected at the 24-hr sampling time; however, the %MN-PCE was significantly increased at the 48-hr sampling time in both males and females at 100 and 200 mg/kg p.o. The sponsor attributed these increases to hypothermia. Body temperature was not measured in the study. However, the sponsor noted that in a pharmacology study, reduced body temperature was observed at doses of 50-200 mg/kg, with the effect lasting from 2-19 hrs at 50 mg/kg, from 2 to 24 hrs at 100, from 2-48 hrs at 200 mg/kg. The sponsor provided no evidence to support a relationship between hypothermia and positive responses in the *in vivo* micronucleus assay.

There have been several published studies investigating the potential role of hypothermia in the formation of micronuclei, most if not all conducted by Asanami and colleagues. Asanami and Shimono [Asanami S, Schimono K. *Mutation Res* 393:91-98, 1997] were apparently the first to demonstrate a causal relationship between hypothermia and induction of micronuclei *in vivo* using reserpine. Reserpine produces prolonged hypothermia, but is negative in the *in vitro* chromosomal aberration assay in mammalian cells [± S9]. Asanami and Schimono [1997] demonstrated that the increases in

micronucleated PCEs [MN-PCE] observed in mice treated with reserpine were prevented by controlling rectal temperature. In that study, MN-PCEs were increased at doses of reserpine that decreased rectal temperature to $<33^{\circ}\text{C}$ for 40 hrs [at sampling times >24 hrs]. When animals were maintained at a higher ambient temperature [30°C (vs 23°C room temperature)] following dosing, no increases in MN-PCEs were observed. It was notable that except for the 96-hr sampling time, the increases in MN-PCE produced by reserpine were not dose-related. No bone marrow cytotoxicity was detected. Asanami and Schimono [1997] demonstrated that the frequency of MN-PCEs containing "relatively large" micronuclei was increased, suggesting (according to the authors) "...that hypothermia disturbs the mitotic apparatus in mammalian cells in vivo". That colchicine produced a similar increase in "relatively large" micronuclei was considered supportive of this hypothesis. Asanami *et al.* [Asanami S, Shimino K, Kaneda S. *Mutation Res* 413:7-14, 1998] conducted a similar experiment using chlorpromazine, an antipsychotic drug which produces transient hypothermia. Asanami *et al* [1998] tested chlorpromazine [CPZ] in an *in vitro* chromosomal aberration assay in CHL and in an *in vivo* micronucleus assay in mice. CPZ was negative in the *in vitro* assay with and without metabolic activation. In the *in vivo* micronucleus assay, CPZ produced increases in MN-PCEs at doses associated with decreases in rectal temperature to 29°C that persisted for at least 11 hrs. [An increase in MN-PCEs was not obtained at a dose that lowered rectal temperature to 29°C for only 7 hrs.] At the 36- and 48-hr sampling times, increases in MN-PCEs were not dose-related; at the 72-hr time, increases were observed only at the HD. [No increases in MN-PCEs were observed at the 24-hr sampling time, and no cytotoxicity was observed at any sampling time.]. As with reserpine, MN-PCEs were not increased when CPZ-treated animals were maintained at higher ambient temperature. Also as with reserpine, "relatively large" micronuclei accounted for much of the increase in MN-PCEs [53-58%], similar to colchicine [56%]. Regarding the relative size of the micronuclei, it should be noted that in neither the reserpine or CPZ study did the authors assess, for comparison, the size(s) of micronuclei induced by a known clastogen. In a subsequent study, Asanami and Schimono [Asanami S, Schimono K. *Mutation Res* 471:81-86, 2000] investigated the relationship between hypothermia and formation of MN-PCEs in Sprague-Dawley rats treated with CPZ and reserpine. Reserpine reduced rectal temperature in rats, but not $<33^{\circ}\text{C}$. No induction of MN-PCEs was observed. CPZ induced hypothermia, and induced MN-PCEs at doses producing decreases in rectal temperature of $\leq 33^{\circ}\text{C}$ for 20 hrs. [Only the HD reduced rectal temperature to that extent, so dose-response could not be evaluated.] With CPZ, increases in MN-PCEs were observed at the 48-hr, but not the 24-hr sampling time. When reserpine-treated animals were maintained in a reduced ambient temperature [16°C] for 24 hrs, rectal temperature was $<33^{\circ}\text{C}$ for 12 hrs; increases in MN-PCEs were obtained. No increases in MN-PCEs were detected in rats maintained at 16°C for <24 hrs. Reserpine produced a relative increase in "large" MN-PCEs, both at the dose associated with a significant increase in MN-PCEs and the next lower dose not associated with increased MN-PCEs. MMC [the positive control] also increased the % "large" MN-PCEs, but to a lesser extent than reserpine. In a more recent study [only the abstract was available at the time of review], Asanami *et al.* [Asanami S, Schimono K, Kaneda S. *J Toxicol Sci* 26(5):323-326, 2001] reported an increase in micronuclei in an *in vitro* chromosomal aberration assay in CHL cells under both hypothermic and hyperthermic conditions. Interestingly, (according to the abstract) the authors noted that "...in CHL cells, hypothermic conditions can induce micronuclei while hyperthermic conditions can induce both chromosome aberrations and micronuclei."

The data from these studies conducted by Asanami and colleagues do suggest that prolonged hypothermia may result in an induction in micronuclei. However, the only other relevant published article obtained through a PubMed search [al-Bekairi AM. *Res Commun Chem Pathol Pharmacol* 73(1):53-64, 1991] reported an opposite effect [although at a much lower ambient temperature than that investigated by Asanami and colleagues], i.e., an inhibition in cyclophosphamide-induced MN-PCEs in Swiss albino mice maintained at 4°C compared to those maintained at 22°C . al-Bekairi [1991] reported that at the 48-hr sampling time, there was an $\approx 50\%$ decrease in MN-PCEs in animals maintained at 4°C

compared to those maintained at 22° C following an acute dose of cyclophosphamide [40 mg/kg i.p.]. Although not statistically significant, the number of MN-PCEs also tended to be lower [47%] at the 24-hr sampling time in animals maintained at 4° C. No protection was observed following a higher dose [80 mg/kg i.p.]. [Body temperature was not measured.]

The *in vivo* micronucleus assays conducted on aripiprazole do not provide sufficient data to dismiss the positive response observed in one of the two studies. In the positive study, doses of 100 and 200 mg/kg were associated with significant increases in MN-PCEs in both males and females. In the negative study, no increase in MN-PCEs was observed at 100 mg/kg [the HD]. Although of note, these data do not necessarily indicate a lack of reproducibility since there may be some inter-assay variability in response. In neither study was body temperature monitored. According to the published data, hypothermia must be at least "mild" [i.e., 30-33° C] and prolonged in order to induce micronuclei. Therefore, it would be critical to accurately quantitate the hypothermia response in order to dismiss the relevance of a positive response. In the studies on CPZ and reserpine, maintaining "normal" body temperature prevented increases in MN-PCEs. A similar study of aripiprazole would provide more convincing evidence of a lack of a clastogenic effect. Strong evidence would be necessary in order to dismiss the positive findings in the *in vivo* micronucleus assay since (a) to this reviewer's knowledge, the relationship between drug-induced hypothermia and induction of micronuclei *in vivo* has not been definitively accepted from a regulatory standpoint and (b) whereas CPZ and reserpine were demonstrated to be negative in an *in vitro* chromosomal aberration assay, aripiprazole was reproducibly positive in *in vitro* chromosomal aberration assay, both without and with metabolic activation. Therefore, based on the data available, aripiprazole should be considered to have clastogenic potential and the *in vivo* positive response should not be dismissed.

Labeling recommendations: the following revisions to the sponsor's proposed labeling are recommended:

Mutagenesis

The mutagenic potential of aripiprazole was tested in the *in vitro* bacterial reverse-mutation assay — ne *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 was clastogenic in — *in vitro* — chromosomal aberration assays in CHL cells — with and without metabolic activation— and in the *in vivo* micronucleus assay in mice.

**APPEARS THIS WAY
ON ORIGINAL**

VI. CARCINOGENICITY

Dose-range finding studies

A number of dose-range finding studies were conducted in both mouse and rat.

Carcinogenicity studies

1. **Study title: 104-week carcinogenicity study of OPC-31 in mice** [Study No. 010379, Volume #1.67-1.70, Conducting laboratory and location: _____, Date of study initiation: _____, GLP, QA'd report:]

Drug, lot #, and % purity: OPC-31, lot no. 93H80M1, purity = _____

CAC concurrence: the original study protocol proposed doses of 0, 1, 3, and 10 mg/kg for males, and 3, 10, and 30 mg/kg for females. mg/kg for 78 wks. The Exe-CAC discussed the protocol in a meeting held on 2/8/94. It was concluded that concurrence could not be given on the doses proposed due to the apparent problems with drug/diet admixture palatability. The committee recommended that gavage dosing be used in the carcinogenicity study, and informed the sponsor that the duration of dosing should be 2 yrs, not 78 wks. The Exe-CAC also recommended that if the sponsor chose to conduct a dietary carcinogenicity study, "...we will not accept it unless there is either histological evidence or reduced survival clearly indicating that an MTD had been achieved... The use of body weight decrease as the sole indication of MTD will not be acceptable." The sponsor responded by conducting a 4-wk palatability study comparing dietary and gavage administration in mice; the sponsor also revised the proposed doses to 0, 1, 3, and 10 mg/kg in both males and females. Based on a review of the 4-wk special study, both the reviewer [Steven S. Sparenborg, Ph.D.] and the Exc-CAC agreed that dietary dosing was acceptable, but recommended doses of 3, 10, and 30 mg/kg in both males and females.

Study Type (2 yr bioassay, alternative model etc.): 2-yr

Species/strain: ICR mouse [CD-1]

Initial age: 6 wks

Initial body wt: 15-25 gm for males, 14-23 gm for females

Number/sex/group; age at start of study: 60/sex/grp

Animal housing: individually

Formulation/vehicle: drug/diet admixture; prepared fresh weekly. Drug concentrations were determined during Wks 76, 77, and 78, and found to be within 10% of intended. Drug concentrations were also tested bi-monthly. Homogeneity was tested and found to be acceptable [data provided].

Drug stability/homogeneity: drug stability was analyzed at the end of the dosing period. As a drug-dietary admixture, drug stability was confirmed for ≤ 17 days when stored at rm temperature.

Methods

Doses: 0, 1, 3, 10 mg/kg

Basis of dose selection: 13-wk dietary dose-range finding study [0, 1, 3, 10 mg/kg]

Restriction paradigm for dietary restriction studies: no

Route of administration: oral

Frequency of drug administration: *ad lib*

Dual controls employed: no

Interim sacrifices: no

Satellite TK: 8/sex/grp

Statistical methods: cf. statistical review.

Observations and times

Clinical signs: animals were observed daily for clinical signs, and twice daily for morbidity or

death. A detailed physical exam, including palpation for masses, was conducted weekly. Body weights: body wts were recorded in main-study and satellite animals prior to the start of dosing, weekly through Wk 16 of dosing, and bi-weekly from Wk 17 on. Body wts of satellite animals were not included in main-study grp means.

Food consumption: food intake was recorded prior to the start of dosing, once a week from Wk 1 through Wk 16, and bi-weekly from Wk 17 on. Food efficiency was calculated for each wk through Wk 13 of dosing.

Hematology: blood samples were collected at the end of the 104-wk dosing period from all main-study survivors for analysis of the following parameters: hct, hgb, rbc ct MCV, MCH, MCHC, platelet ct, wbc ct. Blood smears were prepared, but not examined.

Clinical chemistry: no

Organ weights: wts of the following organs were recorded in 10/sex/grp: brain, pituitary [following fixation], heart, lungs, liver, kidneys, spleen, adrenal glands, testes/ovaries, seminal vesicles, coagulating glands/uteri, prostate].

Gross pathology: a complete necropsy was performed on all main-study animals.

Histopathology: the following tissues were examined microscopically in all animals [to the extent possible]: brain ["3 points"], spinal cord [cervical, thoracic, lumbar], sciatic nerves, pituitary, thymus [or thymic part], thyroid glands, parathyroid glands, adrenal glands, spleen ["two points"], bones and bone marrow [sternum, single femur, vertebrae], knee joint, lymph nodes [cervical, mesenteric], heart ["two points"], aorta, salivary glands, tongue, esophagus, stomach [forestomach, glandular stomach], liver["two points"], gallbladder, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, lungs/bronchi, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles and coagulating glands, ovaries, uterus [uterine horns, cervix], vagina, eyeballs, harderian glands, musculus triceps surae, skin, mammary glands [ventral], gross lesions.

Toxicokinetics: blood samples were collected from satellite animals at 9:00 a.m. at the end of Wks 2 and 52 [3/sex/grp/time point], and from main-study animals [3/sex/grp; no Cs] at 9:00 a.m. at the end of the dosing period for analysis of plasma exposure [conducted by the sponsor].

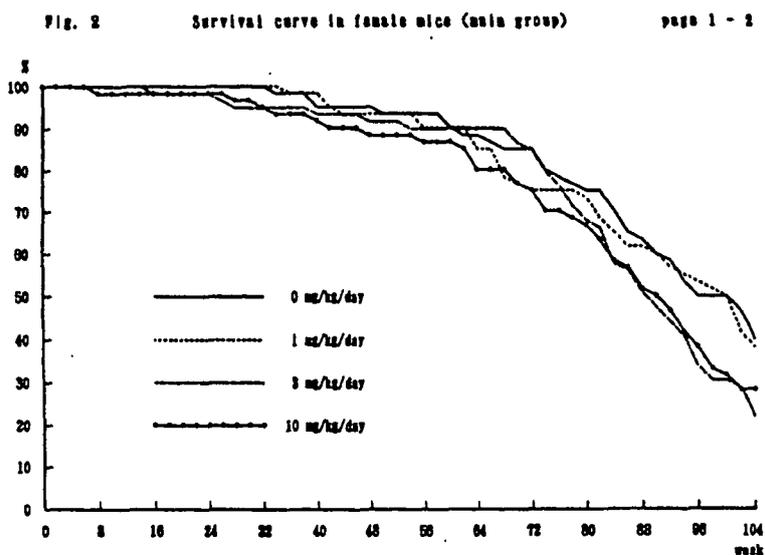
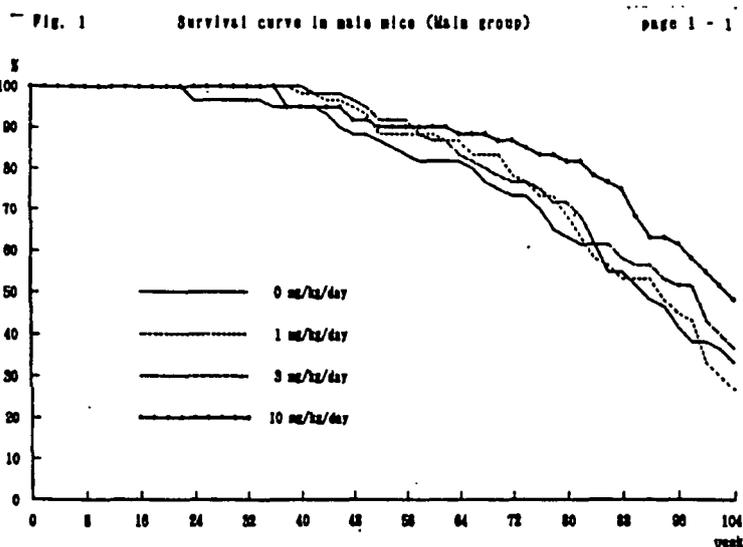
Results:

Mortality: there was no significant increase in mortality rate in any dose grp. The number of survivors in each grp at the end of the dosing period was summarized in the following sponsor's table:

Dosage (mg/kg/day)	Mortality at the end of the administration period	
	male	female
0	40/60 (67)	36/60 (60)
1	44/60 (73)	37/60 (62)
3	38/60 (63)	46/59 (78)
10	31/60 (52)	43/60 (72)

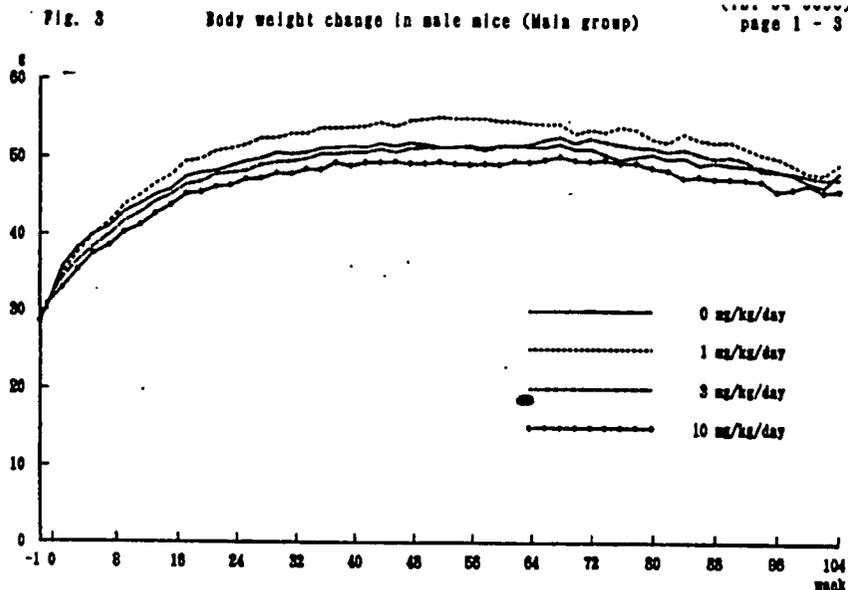
The number (n/n) represents the number of decedents and moribund sacrifices/total number of animals. The number in the parentheses represents mortality (%).

Cumulative survival was illustrated in the following sponsor's figures:

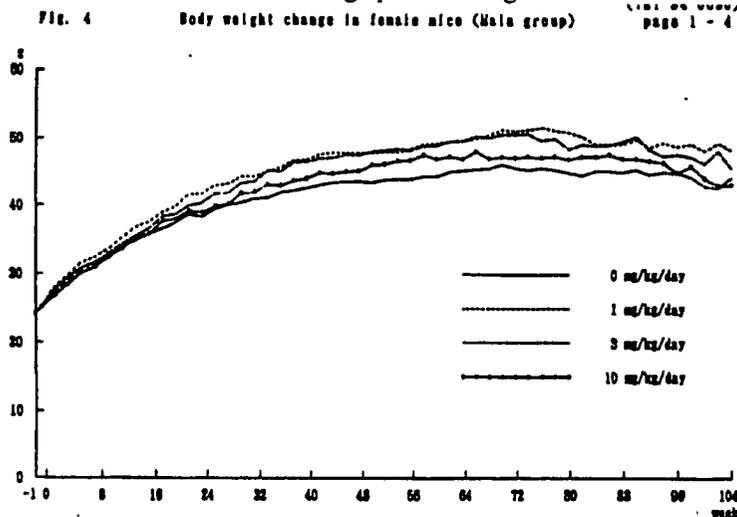


Clinical signs: there were no clear drug-related changes in behavior, although there was a tendency for the incidence of decreased SMA to be higher in HDF [10/60 CF, 10/60 LDF, 11/59 MDF, 18/60 HDF]. The incidence of skin/subcutis masses was greater in MDF and HDF [8/60 CF, 16/60 LDF, 28/59 MDF, 30/60 HDF].

Body weights: in males, mean body wt was transiently decreased at the MD [$\leq 5\%$, primarily during Wks 1-7] and HD [$\leq 8\%$, primarily during Wks 1-40] and transiently increased at the LD [$\leq 8\%$, primarily during Wks 22-78] when compared to CM; final mean body wts were similar among grps. The data are illustrated in the following sponsor's figure:



In females, mean body wt was significantly increased at the LD [throughout most of the dosing period; maximum increase: 15%] and MD [throughout most of the dosing period; maximum increase: 13%], and tended to be higher at the HD when compared to CF. However, by the end of the dosing period, mean body wt was similar among grps. The data are illustrated in the following sponsor's figure:



Summary body wt gain data were not provided.

Food consumption: compared to CM, food intake was significantly reduced in MDM [4-15%] and HDM [4-22%] during the first 20-28 wks of dosing; the greatest effect was at the HD during the first 2 wks of dosing. Food intake was also significantly reduced in LDM [0-11%], primarily during the first 9 wks of dosing, but tended to be increased compared to CM from Wk 64 on. The average daily food intake over the entire dosing period was similar among grps [4.8, 4.9, 4.6, and 4.6 gm in CM, LDM, MDM, and HDM, respectively]. In females, food intake was reduced at all doses during the first 1-4 wks of dosing [LDF: 7% at Wk 3, MD: 8-12% at Wks 2-4, HD: 10-17% at Wks 1-4]. Thereafter, food intake was only sporadically differed from CF. The average daily food intake over the entire dosing period was fairly similar among grps [4.6, 4.7, 4.8, and 4.5 gm in CF, LDF, MDF, and HDF, respectively]. Overall average food efficiency was

similar among grps in both males and females. The sponsor considered the decreases in food intake to be a toxic drug-related effect, rather than a palatability issue since both dietary and gavage administration have been shown to produced decreases in food intake.

Actual doses: ranges of calculated daily doses delivered were as follows: 0.900-1.180, 2.76-3.32, 8.06-11.55 mg/kg for LDM, MDM, and HDM, respectively, and 0.825-1.139, 2.49-3.57, and 7.71-12.08 mg/kg for LDF, MDF, and HDF, respectively. Mean daily doses were as follows: 1.002, 3.01, and 10.04 mg/kg for LDM, MDM, and HDM, respectively, and 1.005, 3.03, and 10.12 mg/kg for LDF, MDF, and HDF, respectively.

Hematology: there were no apparent drug-related findings.

Organ weights: in males, the primary findings were decreases in absolute and relative wt of seminal vesicles at the MD [13-10%] and HD [46-39%] and in prostate at the MD [21-18%] and HD [28-21%]. Absolute and relative wts of lung [9-54%] and liver [18-40%] were increased [only significant for relative liver wt at MD] at all doses; however, the effects were not dose-related.

In females, the primary finding was a marked increase in pituitary wt [absolute and relative] at all doses [2.8-2.9, 2.7-2.6, and 11.4-12.3 fold at LD, MD, and HD, respectively. Additional findings in females consisted of the following: (a) increases in absolute and relative lung wt [18-17, 36%, and 35-45% at LD, MD, and HD, respectively; significant only at MD], (b) increases in absolute and relative liver wt [31-22, 40-35, and 19-23% at LD, MD, and HD, respectively; significant only at MD], (c) decreases in absolute and relative ovary wt [76, 70, and 84-82% at LD, MD, and HD, respectively; not significant], and (d) decreases in absolute and relative uterus wt [50-57, 83-82, and 86% at LD, MD, and HD, respectively; significant at MD and HD].

Gross pathology: the sponsor summarized the findings in survivors, animals dying or sacrificed moribund, and all animals (per sex) in the following tables:

Survivors

Group	Findings	Dose group (mg/kg/day (No. of examined animals))							
		Male				Female			
		0(20)	1(16)	3(22)	10(29)	0(24)	1(23)	3(13)	10(17)
External appearance	Emaciation	0	0	0	0	0	0	0	0
Skin/subcutis	Hair loss	6	1	2	3	6	7	1	4
	Mass(es)	2	2	2	2	4	6	10†	8†
Pituitary	Enlargement	0	0	0	1	0	1	6†	2
	Mass(es)	0	0	0	0	1	4	2	10†
Lungs	Mass(es)	10	7	10	11	12	9	8	7
Abdominal cavity	Ascites	1	0	2	1	3	0	1	1
Liver	Spot(s)	0	1	3	0	0	1	1	2
	Mass(es)	7	10	11	19†	4	3	0	3
Spleen	Enlargement	1	0	3	6	4	5	6	3
Kidneys	Pelvic dilatation	5	1	0‡	0‡	0	0	0	0
Urinary bladder	Distended with urine	2	3	0	2	0	0	0	0
Seminal vesicles	Distention	12	9	5‡	9‡				
Congulating glands	Distention	11	8	5‡	9				
Ovaries	Cyst(s)					14	8	6	5
	Hematomas					6	3	2	1
	Mass(es)					3	1	0	0
Uterus	Cyst(s)					0	2	3†	2
	Thickening of wall					13	6‡	2‡	1‡
	Luminal dilatation					0	0	1	0

Each number represents the number of animals with lesions.

†† p<0.05, † p<0.01: statistically significant difference (Fisher's exact probability test)

Decedents and moribund sacrifices

Organ	Findings	Dose group (mg/kg/day (No. of examined animals))							
		Male				Female			
		0(40)	1(44)	3(38)	10(31)	0(36)	1(37)	3(46)	10(43)
External appearance	Emaciation	8	7	6	7	9	2↓	12	10
Skin/subcutis	Hair loss	7	8	3	2	12	6	14	6↓
	Mass(cs)	4	3	2	4	4	8	18↑	24↑
Pituitary	Enlargement	0	0	0	0	0	1	5	6↑
	Mass(cs)	0	0	0	0	2	1	2	3
Lungs	Mass(cs)	14	12	9	16	7	5	19↑	15
Abdominal cavity	Ascites	1	1	5	0	2	8↑	5	5
Liver	Spot(s)	2	0	3	1	2	1	5	7
	Mass(cs)	14	20	25↑	16	2	8↑	9	3
Spleen	Enlargement	14	18	16	6	20	20	16↓	19
Kidneys	Pelvic dilatation	7	5	1↓	1	1	0	2	1
Urinary bladder	Distended with urine	21	20	11↓	11	2	0	2	1
Seminal vesicles	Distention	12	17	8	4				
Coagulating glands	Distention	11	15	8	5				
Ovaries	Cyst(s)					17	14	14	10↓
	Hematomas					3	3	1	1
	Mass(cs)					2	5	4	0
Uterus	Cyst(s)					0	0	1	1
	Thickening of wall					7	1↓	1↓	3
	Luminal dilatation					6	0↓	1↓	0↓

Each number represents the number of animals with lesions.

↓ p<0.05, ↓↓ p<0.01: statistically significant difference (Fisher's exact probability test)

All animals

Organ	Findings	Dose group (mg/kg/day (No. of examined animals))							
		Male				Female			
		0(60)	1(60)	3(60)	10(60)	0(60)	1(60)	3(60)	10(60)
External appearance	Emaciation	8	7	6	7	9	2↓	12	10
Skin/subcutis	Hair loss	13	9	5↓	5↓	18	13	15	10
	Mass(cs)	6	5	4	6	8	14	28↑	32↑
Pituitary	Enlargement	0	0	0	1	0	2	11↓	8↑
	Mass(cs)	0	0	0	0	3	5	4	13↑
Lungs	Mass(cs)	24	19	19	27	19	14	27	22
Abdominal cavity	Ascites	2	1	7	1	5	8	6	6
Liver	Spot(s)	2	1	6	1	2	2	6	9↑
	Mass(cs)	21	30	36↑	35↑	6	11	9	6
Spleen	Enlargement	15	18	19	12	24	25	22	22
Kidneys	Pelvic dilatation	12	6	1↓	1↓	1	0	2	1
Urinary bladder	Distended with urine	23	23	11↓	13↓	2	0	2	1
Seminal vesicles	Distention	24	26	13↓	13↓				
Coagulating glands	Distention	22	23	13	14				
Ovaries	Cyst(s)					31	22	20↓	15↓
	Hematomas					9	6	3	2↓
	Mass(cs)					5	6	4	0↓
Uterus	Cyst(s)					0	2	4	3
	Thickening of wall					20	7↓	3↓	4↓
	Luminal dilatation					6	0↓	2	0↓

Each number represents the number of animals with lesions.

↓ p<0.05, ↓↓ p<0.01: statistically significant difference (Fisher's exact probability test)