

and post-implantation lethality and external abnormalities. Non-fertile or non-copulated F1 males were necropsied and examined for presence of sperm in the epididymis.

Results: Perinatal and postnatal study of OPC-14597 administered orally to rats

Parameter	Observed Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)
Maternal Toxicity			
Body weight	Decrease* at end gestation through post partum d 21 (30 mg/kg)	10	30
Body weight gain	Decrease* at end gestation at 30 mg/kg Increase* post partum through d. 21 at 30 mg/kg	10	30
Food consumption	Decrease* at end gestation through post partum d 21 (30 mg/kg)	10	30
	Increase (*at 3 and 10 mg/kg/day)	<3	3
Clinical signs	No effect	30	>30 ¹
Mortality	No effect	30	>30 ¹
Duration of gestation	Prolonged*(slightly)	10	30
Lactation	Poor "nursing behavior" and "post-partum care"	10	30
F1 at birth			
Stillbirths	Increase (*at 30 mg/kg/day)	10	30
External abnormalities	No effect	30	>30 ¹
Sex ratio, Male: Female	Increased (*at 10 mg/kg/day only ²)	3	10 ²
F1 Postnatal developmental effects			
Survival Rate	Decrease* (up to postnatal day 4 at 30 mg/kg/day)	10	30
Altered body weight	Decrease*(30 mg/kg/day)	10	30
Physical development	Slight delay (ns) in incisor eruption and eyelid opening	10	30
Neurobehavioral	- Reflexes	No effect	30
	- Learning ability	Slight decrease (ns) in conditioned avoidance ratio	10
F1 Reproductive capacity			
Fertility (% pregnant)	No effect	30	>30 ¹
# days until mating	No effect	30	>30 ¹
F1 maternal weight	Decrease * (30 mg/kg/day)	10	30
N corpora lutea	Slight decrease (ns) (30 mg/kg/day)	10	30
N implants	Slight decrease (ns) (30 mg/kg/day)	10	30
F2 pre-implantation loss	Slight increase (ns) (30 mg/kg/day)	10	30
F2 post-implantation loss	Slight increase (ns) (30 mg/kg/day)	10	30
F2 N live fetuses	Slight decrease (ns) (30 mg/kg/day)	10	30
F2 external anomalies--	No effect	30	>30 ¹
SUMMARY			
F0 Maternal toxicity	Decreased food consumption throughout treatment (up to postpartum day 21) & lower weight gain at end- pregnancy	10	30
F1 Prenatal toxicity	Increased stillbirths	10	30
	Increased male: female sex ratio (?)	3	10 (?)
F1 Postnat. development	Decreased viability and weight gain	10	30
F1 Reproduction	Decreased F1 weight in pregnancy	10	30

¹ Statistically significant; n.s. = Not statistically significant; ² LOAEL not reached (highest dose tested: 30 mg/kg/day); The apparent lack of dose-dependence might be due to the high number of dead offspring at the highest dose, whose gender was not determined e.g. at 30 mg/kg/d, 44 pups from 14 litters were dead at birth, compared to zero in control); NA= not applicable; ND = not determined

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY SUMMARY

The following animal reproduction studies were performed (daily gavage doses in mg/kg in parentheses):

Segment I. Fertility and general reproductive performance:

1. Fertility and general reproductive performance study of OPC-14957 administered orally to rats (2, 6, 20)
2. Male fertility study of OPC-14957 administered orally to rats (20, 40, 60)

Segment II. Prenatal embryofetal development:

3. Preliminary teratogenicity study of OPC-14957 administered orally to rats (2, 6, 20, 30)
4. Teratogenicity study of OPC-14957 administered orally to rats (3, 10, 30)
5. Supplemental teratogenicity study of OPC-14957 administered orally to rats (30 mg/kg/day)
6. Preliminary study (I) to oral teratogenicity study of OPC-14957 in rabbits (non-pregnant) (10, 30, 60)
7. Preliminary study (II) to oral teratogenicity study of OPC-14957 in rabbits (pregnant) (10, 30, 100)
8. Teratological study of OPC-14957 administered orally to rabbits (10, 30, 100)

Segment III. Perinatal and postnatal studies:

9. Preliminary peri/postnatal study of OPC-14957 administered orally to rats (1, 3, 10, 20)
10. Preliminary peri/postnatal study of OPC-14957 administered orally to rats (II)(30 mg/kg/day)
11. Perinatal and postnatal study of OPC-14957 administered orally to rats (3, 10, 30)

Out of the listed 11 studies, 5 were preliminary (dose finding), and 6 were full-scale studies, performed in compliance with the GLP requirements (including 2 fertility studies (rat), 3 teratogenicity studies (2 in rats and 1 in rabbits), and 1 peri/postnatal study (rat). All rat studies were performed in the Sprague Dawley strain; the rabbit studies were carried out in the White New Zealand strain. Multiple full-scale Segment I and Segment II studies were performed in the rat for the following reasons: 1). A second fertility study with higher doses was needed to assess the male reproductive effects, not sufficiently expressed in the first Segment I study; and 2). A supplemental teratogenicity study, replicating the high dose group of the original Segment II study was carried out to determine the reproducibility of some specific developmental effects observed at that dose in the original study.

Segment I. Fertility and general reproductive performance

Exposure of *male and female* rats to 2, 6, and 20 mg aripiprazole /kg/day for 9 weeks prior to and during mating (males) and for 2 weeks prior to mating through gestation day 7 (females), did not cause general toxicity or impaired fertility in the parental generation, neither congenital anomalies in the progeny (F1) up to the highest dose. However, significant and dose-dependent estrus cycle irregularities were induced in F0 females at all dose levels, including the lowest tested dose (2 mg/kg/day), along with a slight but significant increase in the number of corpora lutea. In pregnancy, maternal weight gain was depressed at the mid- and high dose (LOAEL= 6 mg/kg/day); increased pre-implantation embryonic loss and decreased fetal weight were observed at these doses (LOAEL = 6 and 20 mg/kg/day, respectively). These effects were mediated through maternal rather than paternal exposure because when treated F0 males were paired with untreated females, similar effects did not occur. The maximum no-effect doses (NOAEL) were as follows: 20 mg/kg/day for male fertility and reproductive performance in view of lack of effect up to the highest tested dose; less than 2 mg/kg/day (NOAEL not reached) for female fertility and reproductive performance in view of the disturbances in estrus cycle at 2 mg/kg/day or higher; 2 mg/kg/day for the maternal general toxicity in view of the effects on body weight and food consumption

in pregnancy; and 6 mg/kg/day for fetal toxicity in view of the decrease in fetal body weight at 20 mg/kg/day.

F0 male-only exposure to higher dose levels (20, 40, and 60 mg/kg/day) produced signs of general toxicity (significant body weight decrease), as well as reduced organ weight and histopathological changes in reproductive organs at the mid- and/or high dose (atrophy of the prostate at the mid- and high dose, disturbances of spermatogenesis, atrophy of seminiferous tubules and decrease of sperm in the epididymal duct at the high dose). Upon pairing with untreated females, however, no changes in fertility, reproductive capacity, or prenatal development of the F₁ generation were observed. The LOAEL for F0 male general toxicity and histopathological changes in male reproductive organs was 40 mg/kg/day; a LOAEL for male fertility and embryo-fetal development was not reached (> 60 mg/kg/day). The NOAEL for these endpoints were 20 and 60 mg/kg/day, respectively.

The only *selective* reproductive adverse effects seen in Segment I studies at parentally non-toxic doses are the female estrus cycle irregularities and the slight but significant increase in the number of corpora lutea (LOAEL = 2 mg/kg/day). The sponsor has attributed these findings to endocrine changes associated with increased prolactin release characteristic for the pharmacological effect of aripiprazole and other antipsychotic drugs in the rat through blocking of central dopaminergic neurotransmission. Although no prolactin measurements were performed in this particular study, the sponsor has conducted investigative studies to evaluate the potential for aripiprazole to alter serum prolactin levels in rodents (See Application Summary, Vol.1, Item 3, p. 111). In a 13-week oral study in rats (10 to 60 mg/kg/day), serum prolactin level increased in females with the greatest increase at the lowest dose, while in the males it increased minimally at 10 mg/kg/day and decreased relative to controls at 40 and 60 mg/kg/day. Prolactin increase was seen in the females even with administration of single doses as low as 1 and 3 mg/kg/day. Persistent diestrus, a female reproductive tract change secondary to prolonged hyperprolactinemia, was observed at dietary doses of 3 mg/kg/day and greater in female mice and rats. The sponsor considers this effect irrelevant to the human because aripiprazole does not increase serum prolactin in humans (see Application Summary, Vol.1, Item 3, p. 113, and Table S.6.4.4.A, Vol. 93, Item 8, page 148). These observations suggest that for this drug, the rat is not an adequate animal model to predict human reproductive and developmental toxicity.

Segment II. Embryo/fetal development

The effect of aripiprazole prenatal exposure on embryo/fetal development was studied in two species after maternal oral administration during the period of organogenesis (gestation day 7-17 in the rat, and 6-18 in the rabbit).

In the rat, dose levels of 3, 10, and 30 mg/kg/day were employed; the highest dose was presumed to cause some maternal toxicity on the basis of the preliminary teratology study. To assess effects manifested in progeny both pre- and postnatally, part of the pregnant females were subjected to Cesarean section at end pregnancy (gestational day 20) and relevant endpoints were measured to assess maternal and embryo/fetal toxicity; the rest were allowed to deliver spontaneously and nurse their offspring until weaning at the age of 21 days. Postnatal viability, physical and neurobehavioral development of the generation (F₁) was followed until maturity (42 days of age). To assess F₁ reproductive capacity, F₁ males were mated with F₁ (non-sibling) females within the treatment groups. F₁ fertility and F₂ prenatal toxicity parameters were studied. F₁ males of the high dose group were subsequently mated again (second pairing at 19-20 weeks of age) with untreated females to assess independently aripiprazole effect on F₁ male reproductive function.

Maternal toxicity was observed at 10 mg/kg/day and higher e.g., decreased food consumption and suppressed weight gain at 10 and 30 mg/kg/day; clinical signs (ptosis, disappearance of touch response,

attributed to a central inhibitory action of the drug) - at 30 mg/kg/day. The period of gestation was slightly but significantly prolonged at the high dose.

Adverse prenatal fetal effects were found at maternally toxic levels (at and/or above 10 mg/kg/day) and included decreased placental weight, decreased fetal weight, retarded skeletal ossification, and increased incidence of visceral abnormalities (high dose), but no effect on embryo-fetal lethality. It should be noted that examination for visceral abnormalities was performed on both fetuses (all groups), and neonatal animals (high dose and control only) culled on postnatal day 4. The observed visceral abnormalities included abnormal shape of the liver, sporadically in combination with diaphragmatic hernia (in neonatal animals, but not in the fetuses); undescended testes was found in the fetuses but not in the neonates. Abnormal shape of the liver (with a nodule on the diaphragmatic side of the liver, sometimes protruding into thoracic cavity) was the most prevalent anomaly. It was seen in neonatal animals from the high dose group on postnatal day 4 (in 8 pups from 3 litters, or 14% of the examined), as well as in 2 pups out of 70 examined from the same group at the age of 6 weeks, but not in the control. In 2 out of the total 10 cases of abnormal shape of the liver, there was an accompanying diaphragmatic hernia. Because these abnormalities were seen only at the highest exposure level, their relation to aripiprazole treatment cannot be excluded. However, this is not likely to be an agent-specific effect because it was reported to occur spontaneously (e.g., in some untreated adult females used in the fertility study, as well as in a control male animal in the Segment III study). These data, as well as the fact that this abnormality did not interfere with the growth and survival (e.g., the offspring with abnormal shape of the liver did not die during the course of the study and showed no marked difference in body weight compared with other animals from the same dose group), suggest that the abnormal shape of the liver can be classified as a variation rather than malformation. Undescended testes were noted in 2% (3 fetuses from 3 litters) of the examined high-dose fetuses at term, but not postnatally; thus, this appears to be a transient delay, unlikely to continue after birth.

Postnatally, body weight gain was suppressed dose-dependently in both male and female offspring at 10 and 30 mg/kg/day (significant at the latter). At the low dose however (3 mg/kg/day), pup body weight was significantly increased; similar effect was also seen in adult animals at low aripiprazole oral doses (6 mg/kg/day) (Segment I study No. 005437, volume 1.109, p. 220). A dose-dependent delay in physical maturation (vaginal opening) was seen in female offspring (significant at 10 mg/kg/day and higher, but also present - although without reaching statistical significance - at 3 mg/kg/day). No delays in other developmental landmarks and no significant changes in neurobehavioral cognitive development of the progeny were found. However, impaired reproductive capacity of the progeny (decreased number of corpora lutea and implantations, slightly increased pre- and post-implantation F2 embryoletality) was induced by prenatal exposures of 10 mg/kg/day and higher. The impaired reproductive capacity of F1 generation was most probably mediated through the F1 prenatally treated females, because such impairment was not seen when untreated females were mated with the prenatally treated F1 males. In order to determine if the decrease in reproductive capacity and the retardation of sexual maturation (vaginal opening), seen in F1 female offspring occur reproducibly, the sponsor conducted a supplemental Segment II study. Only one treatment dose was employed, equal to the high dose (30 mg/kg/day) that, in the previous study, had induced significant changes in these parameters. The route of administration and the period of exposure were identical to the previous experiment. The supplemental study (which used a lot of a slightly higher purity, and was performed in a different year and season) showed less maternal toxicity and fetal effects. However, it confirmed the retardation in F1 vaginal opening, as well as the retardation in F1 physical development as demonstrated by a consistently lower body weight of both the male and female offspring through maturity. Nevertheless, there were no significant changes in the reproductive performance of F1 generation in this experiment (there was a trend to a decreased number of implantations and live fetuses, and of increased pre-implantation embryonic loss but these effects did not reach statistical significance).

In summary, the lowest adverse effect- and highest no-effect levels in the Segment II rat studies were as follows:

Maternal toxicity: LOAEL 10 mg/kg/day; NOAEL 3 mg/kg/day

Prenatal developmental toxicity: LOAEL: 10 mg/kg/day (retarded fetal skeletal ossification) and 30 mg/kg/day (reduction in placental weight). Both these doses are maternally toxic. Visceral abnormalities are seen postnatally in the progeny at 30 mg/kg/day, but LOAEL and NOAEL for this effect cannot be determined because this endpoint was not examined postnatally in the lower dose groups.

Postnatal development of progeny: (deviations in postnatal weight gain, delayed sexual maturation in the female offspring). LOAEL: 3 mg/kg/day; NOAEL: <3 mg/kg/day.

Note: We estimate LOAEL at 3 mg/kg/day (maternally non-toxic dose) in view of the dose-dependent retardation in F1 female sexual maturation. The effect is significant at the high and middle dose (10 and 30 mg/kg/day), but it is also present at the low dose as a trend to a selective delay in vaginal opening, in contrast to the higher body weight and the accelerated development of other physical landmarks (incisor eruption and eyelid opening) at this dose. This assessment is different from the sponsor's LOAEL for postnatal development (10 mg/kg/day).

Effect on the reproductive capacity of F1 generation: (decreased number of corpora lutea and implantations, slightly increased pre- and post-implantation F2 embryoletality).

First study: LOAEL: 10 mg/kg/day; NOAEL: 3 mg/kg/day;

Supplemental study: LOAEL: 30 mg/kg/day; NOAEL: not determined.

Comments: The results from the Segment II studies in the rat show that prenatal exposure to aripiprazole during embryogenesis induces adverse *prenatal* effects only at doses that are maternally toxic (at or above 10 mg/kg/day). However, it appears to affect *selectively* the *postnatal* sexual maturation of the *female* progeny down to a dose level that is not maternally toxic (3 mg/kg/day); a trend to female-mediated decrease in the reproductive capacity of the progeny is seen at higher doses (10 and/or 30 mg/kg/day). These results are supported by the preceding Segment I study (see previous page) in the sense that in both F0 and F1 generation, the females were more susceptible than the males to the adverse effects of aripiprazole on reproduction. These adverse effects, affecting both F0 (estrus cyclicity) and F1 (embryo/fetal development, sexual maturation and reproductive capacity), were female-mediated. According to the sponsor, these effects may be attributable to the ability of the drug to increase prolactin levels in the rat. Their relevance to humans is questionable having in mind that aripiprasole did not increase prolactin in humans, either males or females (see Table S.6.4.4.A, Vol. 93, Item 8, page 148).

In the rabbit, the Segment 2 study employed the dose levels of 10, 30, and 100 mg/kg/day from day 6 to 18 of gestation. The highest dose was shown to produce signs of maternal toxicity (a decrease in food consumption) in a preliminary study. All pregnant animals were subjected to caesarean on gestation day 28. Endpoints evaluated included maternal general condition, body weight, food consumption, clinical signs and mortality, number of corpora lutea, implantations, embryo/fetal death, placental and fetal weight of live fetuses, fetal gender, and external, visceral and skeletal anomalies, as well as maternal gross pathology.

A significant, dose-dependent decrease in maternal food consumption was induced at all dose levels but no decrease in maternal body weight was present, except as a trend at the high dose. Placental weight decrease and embryo/fetal mortality occurred only at the high dose; fetal weight was decreased at the mid-dose, 30 mg/kg/day (males only) and at the high dose, 100 mg/kg/day (significant in both males and females). Treatment-related visceral anomalies were not observed, but a dose-dependent increase was found in skeletal variations and skeletal abnormalities (fused sternbrae). The latter were significantly increased at the highest dose, involving 30% (25 of 82) fetuses and 60% of the litters examined, but were

also present in the 30 and 10 mg/kg/day groups at rates of 3% (4/128) and <1% (1/141) of fetuses, respectively. Although such anomalies are reported to occur spontaneously (literature data), none were found in the concurrent control group. Their dose-dependence and high incidence in the high dose group support their relationship to treatment.

Based on these findings, the LOAEL for maternal toxicity in the rabbit is 10 mg/kg/day (in view of the significantly decreased food consumption), and the LOAFL for fetal toxicity is 30 mg/kg/day (in view of increased incidence of skeletal anomalies and fetal weight decrease). NOAEL for general maternal toxicological effect was not reached (<10 mg/kg/day, the lowest tested dose) and the NOAEL for embryo/fetal development was 10 mg/kg/day. There is no evidence for a selective embryo/fetal effect on embryo/fetal development in this species.

Segment III. Perinatal and postnatal studies

In these studies, performed in the rat only, pregnant F0 females were treated with aripiprazole (3, 10, and 30 mg/kg/day) peri- and postnatally (from gestation day 17 to post-parturition day 21). The animals were allowed to deliver spontaneously and nurse their progeny up to postnatal day 21; maternal general condition, body weight, food consumption, parturition and lactation were evaluated. On postnatal day 4, the litters were culled to a standard number of 8 (4 per sex where possible). Maternal animals were sacrificed on p.n. day 22; the number of implantation sites was counted. The progeny was examined at birth (live and dead newborns, body weight, external abnormalities) and postnatally until day 42 of life; the endpoints included F1 postnatal physical development (weight, developmental milestones), neurobehavioral development (reflex function, learning (males only), and sexual maturation. F1 reproductive performance was examined at the age of 11-13 weeks, when F1 males and females (non-sibling) were paired within the dose groups to produce F2 generation. F1 maternal weight and food consumption were measured during gestation; Cesarean section was performed at end gestation (day 20), and indices of F2 prenatal development were measured (corpora lutea, placental weight, and pre- and post-implantation lethality and external abnormalities). Non-fertile or non-copulated F1 males were necropsied and examined for presence of sperm in the epididymis.

F0 maternal toxicity (decreased food consumption and body weight at end pregnancy and post partum) was induced at the high dose along with slightly but significantly prolonged gestation, and poor lactation. After birth, despite of the continued dosing through lactation, a dose-dependent increase in maternal weight gain took place, significant at the high dose.

Significant adverse effects in *F1 pre- and postnatal development* (increased stillbirths, decreased postnatal survival, and inhibition of body weight through adolescence) occurred at the high dose. However, the surviving progeny showed no significant differences in the neurobehavioral and physical development (except for a slight delay in incisor eruption at the high dose, most likely as a consequence of the lower F1 body weight). The F1 reproductive performance was slightly but consistently affected at the high dose: non-significant decreases in corpora lutea (mean 14.7 vs. 15.6 in the control), implantations (13.8 vs. 14.9), live fetuses (12.7 vs. 14.3), and increase in early resorptions (7.6 vs. 3.7) were observed.

The LOAEL is 30 mg/kg/day for both mothers and offspring; there are no selective effects on the progeny. The NOAEL is 10 mg/kg/day.

Table
Summary of reproductive and developmental toxicity findings

Parameter	Observed Effect	NOAEL (mg.kg/day)		LOAEL (mg.kg/day)	
		Males	Females	Males	Females
Segment I: Fertility and general reproductive performance (Rat)					
Parental male and female exposure (pre-mating and early pregnancy)					
F ₀ General Parental toxicity	Altered body weight & food consumption*	2	2	6	6
F ₀ Maternal toxicity	Decreased* weight gain in pregnancy	NA	2	NA	6
F ₀ Fertility and reproductive performance	Females: Estrus cycle prolongation & irregularity*; Hyperovulation* (Males – no effect)	20	<2 ²	>20 ¹	2
F1 Embryo/fetal effects	Increased pre-implantation lethality*	20	2	>20 ¹	6
	Decreased fetal weight*	20	6	>20 ¹	20
Parental male-only exposure (pre-mating)					
F ₀ Male general toxicity	Decreased body weight *	<20 ²	NA	20	NA
F ₀ Male reproductive organs	Prostate atrophy	20	NA	40	NA
F ₀ Spermatogenesis	Disturbed	40	NA	60	NA
F ₀ Male Fertility and reproductive performance	No effect	60	NA	>60 ¹	NA
F1 Embryo/fetal effects	No effect	60	NA	>60 ¹	NA
Segment II: Effect on embryo-fetal development (exposure in pregnancy during organogenesis)					
A). Rat					
Maternal toxicity					
- Main Study	Decreased food consumption & weight (10 (n.s) and 30* mg/kg/day)	3		10	
	Clinical signs	10		30	
- Supplemental Study	Slight decrease maternal weight (n.s.)	ND		30 ³	
F1 Embryo/fetal effects					
- Main Study	Retarded skeletal ossification: 10 (n.s) 30* mg/kg/day	3		10	
	Decreased placental & fetal wt; undescended testes	10		30	
	Visceral anomalies:				
	- diaphragmatic hernia (fetuses, neonates)	10		30	
	- abnormal liver shape (not found in fetuses, only in neonates)	ND (lower dose groups not examined for visceral anomalies in neonates)		30 ³	
- Supplemental Study	Decreased birth weight (n.s.); (visceral & skeletal examination not performed)	ND		30 ³	
F1 Postnatal effects					
- Main Study	Altered body weight & weight gain, Delayed vaginal opening : 3 (n.s) 10*, 30* mg/kg/d	<3 ²		3	
- Supplemental Study	Decreased wt. gain , Delayed vaginal opening (n.s.)	ND		30 ³	
F1 Reproductive capacity (prenatally treated males & females)					
- Main Study	Decrease in: N corpora lutea, implantations, maternal weight gain pregnancy (10, 30* mg/kg/day); Increased embryonic loss (n.s.)	3		10	
		10		30	
- Supplemental Study	Decreased N implantations (n.s.), Increased embryonic loss (n.s.)	ND		30 ³	
F1 Male fertility (prenatally treated males & untreated females)					
	Decreased fertility (n.s.), Increased embryonic loss (n.s.)	ND		30 ³	

B). Rabbit			
Maternal toxicity	Decreased food consumption * (dose-dependent)	<10 ²	10
	Decreased weight, slightly (n.s.)	30	100
Embryo/fetal effects:			
Fetal death	Increase (*100 mg/kg/day, n.s. 10 & 30 mg/kg/day)	10	30
Skeletal anomalies	Increased rates of minor anomalies (sternobral fusion) and variations (extra or rudimentary ribs and vertebrae (*100 mg/kg/day, n.s.10 & 30 mg/kg/day)	10	30
Fetal weight	Decrease * (dose-dependent)	10	30
Placental weight	Decrease (*100 mg/kg/day)	30	100
Segment III: Peri-/Postnatal Effects (Rat) (exposure in late pregnancy and through lactation)			
F0 Maternal toxicity	Decreased* food consumption & weight gain at end- pregnancy through postpartum day 21	10	30
F1 Prenatal toxicity	Increased* stillbirths	10	30
F1 Postnatal developmental effects	Decreased* early postnatal survival Decreased* weight gain	10	30
F1 Reproduction	Decreased* F1 maternal weight No effect on fertility	10	30

* Statistically significant; n.s.=non significant ¹LOAEL not reached; ² NOAEL not reached; ³ Only one dose studied; NA= not applicable; ND= not determined

Reproductive and Developmental Toxicology Conclusions

Fertility and general reproductive performance (rat). Upon exposure of sexually mature male (throughout the cycle of spermatogenesis) and female rats (for about 3 estrus cycles), aripiprazole affects selectively the female reproductive function, inducing estrus cycle irregularities and ovulation disturbances down to the lowest tested dose, 2 mg/kg/day (no-effect level not reached). This dose is below the LOAEL for general toxicity in rat female, and on a mg/m² basis, represents about two-thirds of the maximal recommended human dose (MRHD = 30 mg/day, or 0.5 mg/kg/day for a 60-kg person). In the male, adverse effects on reproductive organs (prostate atrophy) and spermatogenesis are induced by much higher levels that are generally toxic (LOAEL = 40 and 60 mg/kg/day, or on a mg/m² basis, 13x and 20x MRHD, respectively). These effects do not impair fertility in either males or females, and, except for a slight (female-mediated) increase in the pre-implantation embryonic loss and a slight decrease in fetal weight, the development of the next (F1) generation is normal. The adverse effects in the female are explained as secondary to aripiprazole-induced hyperprolactinemia specific for the female rat, and are not likely to be relevant to the human because the drug does not increase serum prolactin in either women or men.

Pregnancy/Embryofetal development (rat, rabbit). Upon exposure in pregnancy during the period of organogenesis, aripiprazole has no selective effect on the prenatal development in the rat. Retarded fetal growth (demonstrated as a retarded skeletal ossification, decreased fetal weight, and retarded testes descent) occur at LOAELs of 10 and 30 mg/kg/day, i.e. at or above the LOAEL for maternal toxicity (10 mg/kg/day). Visceral anomalies (diaphragmatic hernia and abnormal liver shape, the latter also seen in some untreated animals) occur at the maternally toxic dose of 30 mg/kg/day. However, the drug affects postnatal development at doses below those inducing maternal toxicity in the rat. Delayed sexual maturation (retarded vaginal opening) of the F1 female offspring is seen at a LOAEL of 10 mg/kg/day and is discernible in single pups even at 3 mg/kg/day. This dose, on a mg/m² basis, is approximately equal to the MRHD. The adequacy of these data for the human is uncertain because the rat is not an appropriate

animal model for predicting the reproductive effects of aripiprazole in humans, having in mind the specific effect of the drug on prolactin in this species, as pointed out above.

In the rabbit, aripiprazole affects embryo/fetal development only at doses that are maternally toxic; therefore the drug is not a selective embryo/fetal toxicant in this species. Spontaneous abortion, lower fetal weight, and an increase in minor skeletal abnormalities are seen at a LOAEL of 30 mg/kg/day, a level corresponding to maternal exposure [AUC (0-24 h)] approximately 3 times that observed at the MRHD in humans. The highest no-effect dose is 10 mg/kg, an exposure [AUC (0-24 h)] over 2 times that observed at the MRHD in humans.

Perinatal and postnatal effects (rat). Upon exposure to aripiprazole in late pregnancy and lactation, a slight but significant delay in parturition, a significantly increased stillbirth rate, decreased early postnatal survival, and decreased weight gain of the progeny are seen at the maternally toxic dose of 30 mg/kg/day (10 times the MRHD based on body surface area). Because aripiprazole is excreted in rat maternal milk, the postnatal developmental disturbances may be due in part to a direct exposure of progeny to the drug. The highest no-effect dose is 10 mg/kg/day – a dose that, based on body surface area, is over 3 times higher than the maximal recommended human dose.

APPEARS THIS WAY
ON ORIGINAL

VIII. SPECIAL TOXICOLOGY STUDIES

Antigenicity/Immunotoxicity

1. OPC-14597 was negative in passive cutaneous and active systemic anaphylaxis assays in male Hartley guinea pigs at doses of 0.5 and 5 mg/kg [4 divided doses: 2 i.m., 2 s.c., once a week for 4 wks]; the challenge dose of OPC-14507 was 10 mg/kg i.v.

2. Study title: **Four-week oral study of T-cell-dependent antibody response in rats** [Study No. DS01005, Conducting laboratory and location: Bristol-Myers Squibb, Syracuse, NY, Date of study initiation: n/s, Report date: 6/01, GLP, QA report: Y]

Drug, lot #, radiolabel, and % purity: BMS-337039, Batch no. C00B92M, assays "as is" = _____
Formulation/vehicle: suspension/5% gum arabic in water

Methods

Dosing: BMS-337039 was administered to Sprague-Dawley rats [10/sex/grp] at doses of 0, 0 [vehicle], 10, 30, and 60 mg/kg for 28 days. Cyclophosphamide was administered to an additional 10/sex [50 mg/kg i.p.] on study Days 25-26 as a positive control. On Day 25, all animals received an i.v. injection of sheep rbc's via tail vein [2×10^8 cells]; blood samples were collected from all animals on Day 29. Animals were sacrificed on Day 29 following blood collection. At necropsy, spleens were weighed and shipped to _____ for analysis of SRBC-specific antibody.

Observations and times: clinical signs, body wt, spleen wt, spleen IgM antibody response to SRBCs [i.e., T-Cell-Dependent Antigen]. Antibody response was assessed using the "modified hemolytic-plaque assay of Jerne", and was expressed as specific activity [AFC/ 10^6 spleen cells; AFC = antibody-forming cells] and total spleen activity [AFC/spleen ($\times 10^3$)].

Results

There were no unscheduled deaths. One animal was removed from the study due to methodological problems. Drug-related clinical signs were observed at 30 and 60 mg/kg, i.e., ptosis, rough/soiled haircoat, chromodacryorrhea, chromorhinorrhea, and dehydration. Mean body wt was reduced in MD and HD males; final mean body wts were 12 and 20%, respectively, lower compared to CM. In females, mean body wt was significantly increased at the LD throughout the dosing period. Final mean body wt in LDF was 12% higher compared to CF; final body wt was not significantly affected at the higher doses in females. Spleen wt was not affected in females. In males, absolute spleen wt was 11% lower in HDM compared to CM; however, body wt-corrected spleen wt was not affected.

In males, there was no significant effect on the antibody response to SRBCs, and the response of water- and vehicle-treated animals was similar. In females, the response in vehicle-treated animals was lower than that in water-treated animals [$\approx 40\%$]. In LDF, the antibody response was significantly increased compared to vehicle-control, but similar to the water-control grp. In MDF, the antibody response was significantly increased compared to both C grps. No effect was observed at the HD. In both males and females, cyclophosphamide produced marked decreases in antibody response [$>90\%$].

Serum Prolactin

The sponsor conducted a number of special toxicology studies in order to determine the effect of aripiprazole on serum prolactin.

1. Study title: Effect of a single oral administration of OPC-14597 on serum prolactin levels in female ICR mice [Study no: 016489, Conducting laboratory and location: Otsuka Pharmaceuticals Inc., Japan, Date of study initiation: 8/29/00, GLP, QA report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. C98G92(2)M, purity: _____
 Formulation/vehicle: suspension/5% gum arabic in water

Methods

Dosing: OPC-14597 was administered to female ICR mice [24/grp] at doses of 0, 3, 10, and 30 mg/kg p.o. Of the 24/grp, 18 were dosed on Day 1 and the remaining 6 were dosed on Day 2. Vaginal smears were obtained prior to treatment in order to determine that animals exhibited a regular estrus cycle and that they were not in proestrus at the time of dosing.

Observations and times: animals were observed prior to dosing and blood collection for general condition. Blood samples were collected for quantitation of serum prolactin levels at 1, 2, and 4 hrs postdosing [8/grp/time point]. Serum prolactin was quantitated using RIA [mouse prolactin]. At sacrifice, a sample of pituitary gland was collected from 1/grp [2 hrs postdosing], but not examined.

Results

Serum prolactin levels were significantly elevated at all time points sampled at all doses. There was no evidence of a dose-response; the magnitude of the effect was similar at all doses. The data are illustrated in the following sponsor's figure:

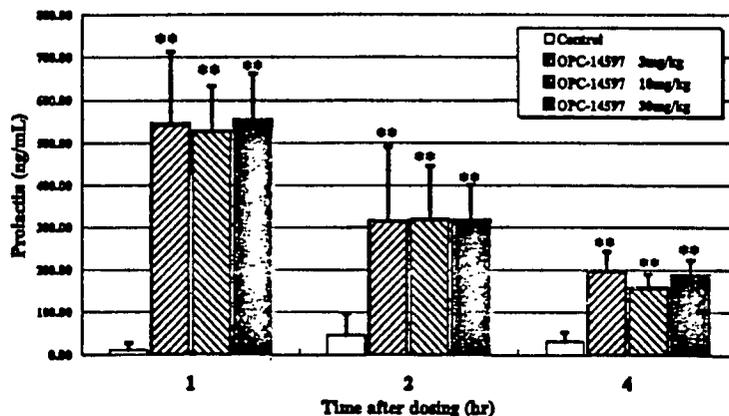


Fig. 1 Effect of a Single Oral Dose of OPC-14597 on Serum Prolactin Levels in Female ICR Mice
 Means indicate mean ± S.D. (number of 8 animals each time point). ** : p < 0.01

Serum prolactin values were highest at 1 hr postdosing at all doses, and ranged from 528.013 ± 112.224 to 553.308 ± 116.103 ng/mL.

2. Study title: Effect of single oral administration of OPC-14597 on serum prolactin level in female rats [Study no: 011332, Conducting laboratory and location: Otsuka Pharmaceuticals Inc., Japan, Date of study initiation: 8/8/95, GLP, QA report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 93H80M1, purity: _____
 Formulation/vehicle: suspension/5% gum arabic in water

Methods

Dosing: OPC-14597 was administered to female Sprague-Dawley rats [6/grp] at doses of 0 and 20 mg/kg p.o. [This dose was selected as the highest dose prolonging diestrus (i.e., pseudopregnancy) in a mating and fertility study.] Vaginal smears were obtained prior to treatment in order to determine that animals exhibited a regular estrus cycle and that they were in proestrus at the time of dosing.

Observations and times: blood samples were collected for quantitation of serum prolactin levels at 1 hr postdosing. Serum prolactin was quantitated using EIA [i.e., enzyme immunoassay method].

Results

Serum prolactin was markedly elevated following administration of OPC-14597 at a dose of 20 mg/kg [9.95 ± 6.40 ng/mL and 252.85 ± 41.98 ng/mL at 0 and 20 mg/kg, respectively].

3. Study title: Serum prolactin levels in female Fischer rats single orally given OPC-14597 [sic] [Study no: 015149, Conducting laboratory and location: Otsuka Pharmaceuticals Inc., Japan, Date of study initiation: 12/16/98, GLP, QA report: Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 98A91M, purity: _____

Formulation/vehicle: suspension/5% gum arabic in water ●

Methods

Dosing: OPC-14597 was administered to female Fischer 344 rats [30/grp] at doses of 0, 3, 10, and 30 mg/kg p.o. Dosing was conducted over 4 days in order to dose every animal during diestrus. Vaginal smears were obtained prior to treatment in order to determine that animals exhibited a regular estrus cycle and that they were in diestrus at the time of dosing.

Observations and times: blood samples were collected for quantitation of serum prolactin levels at 1, 2, and 4 hrs postdosing [10/grp/time point]. Serum prolactin was quantitated using EIA [rat prolactin].

Results

Serum prolactin levels were significantly elevated at all doses, with the maximum effect observed at 1 hr postdosing [all doses]. The data were summarized and illustrated in the following sponsor's figure and table:

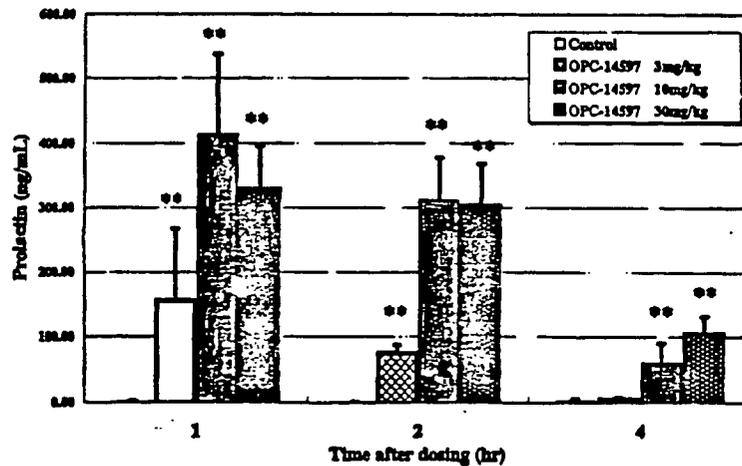


Fig. 1 Serum Prolactin Levels of Female F344 rats single oral treated with OPC-14597
 Bars indicate mean ± S.D. consisted of 10 animals each time point. **: p < 0.01

Table 1
Serum Prolactin Levels in Female Fischer Rats Single Orally Given OPC-14597

Item : Summary of Serum Prolactin Levels	Unit : ng/mL		
	Time after dosing(hr)		
Group	1	2	4
A 0	0.92 ± 1.06	0.80 ± 0.42	2.64 ± 2.87
A 3	155.69 ± 111.98**	74.84 ± 12.72**	5.86 ± 2.21
A 10	411.97 ± 125.93**	310.54 ± 67.58**	57.95 ± 32.79**
A 30	328.64 ± 66.76**	303.18 ± 65.01**	105.07 ± 26.01**

Results indicate mean ± S.D. consisted of 10 animals each time point.

** : p<0.01 Significant difference from the control

4. Study title: Effect of 1-week dietary administration of OPC-14597 on serum prolactin levels in female mice [Study no: 014047, Conducting laboratory and location: Otsuka Pharmaceuticals Inc., Japan, Date of study initiation: 3/9/98, non-GLP, QA report:N]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 93H80M1, purity: _____

Formulation/vehicle: dietary for both OPC-14597 and haloperidol

Methods

Dosing: OPC-14597 was administered to female ICR mice [6/grp] at doses of 0 and 30 mg/kg; haloperidol was administered to an additional 6 females at a dose of 5 mg/kg p.o. [The sponsor noted that haloperidol was associated with mammary gland and pituitary tumors at a dose of 5 mg/kg.] Dosing was for 1 wk.

Observations and times: body wt and food consumption were recorded prior to start of dosing and daily during the dosing period. Blood samples [trunk blood] were collected at 10:00-11:00 a.m. at sacrifice following 1 wk of treatment. Serum prolactin was quantitated using ELISA [rabbit anti-mouse prolactin]. Ovaries, uterus, and vagina were collected and preserved in 10% neutral-buffered formalin and prepared for microscopic evaluation [paraffin-embedded, H & E stain] in order to determine the stage of estrus.

Results

Body wt and food consumption were transiently reduced [8-10 and 30-58%, respectively] with OPC-14597 and haloperidol. The achieved mean dose was 37.2 mg/kg/day for OPC-14597 and 5.8 mg/kg/day for haloperidol. Serum prolactin levels were elevated, but not significantly, in OPC-14597- and haloperidol-treated animal: 21.82 ± 16.67, 192.17 ± 208.13, and 181.13 ± 100.59 ng/mL for C, OPC-14597, and haloperidol, respectively. Serum prolactin levels were higher than C values in 4/6 OPC-14597- treated and 6/6 [markedly in 4/6] haloperidol-treated animals. Both OPC-14597 and haloperidol were associated an increased number of females in persistent diestrus [1/6 C, 5/6 OPC-14597, and 4/6 haloperidol].

5. Study title: Effect of 4-week dietary administration of OPC-14597 on serum prolactin levels in ICR mice [Study no: 015176, Conducting laboratory and location: Otsuka Pharmaceuticals Inc., Japan, Date of study initiation: 1/14/99, GLP, QA report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 98A91M, purity: _____

Formulation/vehicle: dietary

Methods

Dosing: OPC-14597 was administered to ICR mice [10/sex/grp] as a drug/diet admixture at doses of 0, 3, 10, and 30 mg/kg for 28-29 days.

Observations and times: animals were observed daily for general condition. Body wts were recorded prior to start of dosing, weekly during the dosing period, and at sacrifice. Food consumption was recorded weekly during the dosing period. Blood samples were collected at sacrifice [trunk blood] [9:40-11:16 a.m.] for quantitation of serum prolactin levels. Serum prolactin was quantitated using ELSIA [rabbit anti-mouse prolactin antibody]. Ovaries, uterus, and vagina were microscopically examined [10% neutral-buffered formalin, H & E] for determined the stage of estrus.

Results

There were no unscheduled deaths during the study. Achieved doses were calculated to be 3.4-3.8, 11.9-12.6, and 37.3-35.8 mg/kg/day in LD, MD, and HD grps, respectively. Mean body wt was reduced in HDM primarily during the first 2 wks of dosing [12-13%]; final mean body wt in HDM was 7% lower than in CM. In HDM, body wt loss was noted during the first wk of dosing, and body wt gain was significantly reduced throughout the rest of the dosing period. Body wt was not significantly affected in females. Food consumption was significantly reduced during the first wk of dosing in HDM [29%]. Serum prolactin levels were significantly reduced in HDM [81.2 ± 35.3, 61.1 ± 16.2, 59.4 ± 8.4, and 47.4 ± 11.3 ng/mL in CM, LDM, MDM, and HDM, respectively]. Serum prolactin levels were not significantly affected in females [73.5 ± 41.1, 76.3 ± 24.6, 76.3 ± 21.7, and 100.4 ± 44.1 ng/mL in CF, LDF, MDF, and HDF, respectively]. Persistent diestrus was observed only in treated females [0/10 CF, 6/9 LDF, 9/10 MDF, 7/9 HDF].

6. Study title: Effect of 13-week dietary administration of OPC-14597 on serum prolactin level in ICR mice [Study no: 016481, Conducting laboratory and location: Otsuka Pharmaceuticals Inc., Japan, Date of study initiation: 8/29/00, GLP, QA report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. C98G92(2)M, purity: —
Formulation/vehicle: dietary

Methods

Dosing: OPC-14597 was administered in the diet to ICR mice at doses of 0, 3, 10, and 30 mg/kg for 4 or 13 wks [15/sex/grp at both sampling times].

Observations and times: animals were observed for general condition daily. Body wts and food consumption were recorded weekly during the dosing period. Vaginal smears were examined for 2 wks prior to blood collects during Wks 4 and 13 for characterization of the estrus cycle [treated grps only]. Blood samples [trunk blood] were collected at the end of Wks 4 and 13 from all animals for quantitation of serum prolactin levels; collection times were 10:00, 18:00, and 2:00 on Days 30-31 or Days 93-94. Serum prolactin was quantitated using RIA. [Data from animals determined to be in proestrus at time of blood collection were not included in mean calculations in order to avoid variations due to non-drug-related increases in serum prolactin characteristic of proestrus.] Ovaries, uterus, and vagina were microscopically examined [10% neutral buffered formalin, H & E stain] in order to determine stage of estrus.

Results

Overall, achieved doses were calculated to be 3.6-4.2, 12.4-12.9, and 38-37 mg/kg/day at LD, MD, and HD, respectively, in animals treated for 4 wks, and 3.1-3.3, 10.7-11, and 32.1-31.6 mg/kg/day at LD, MD, and HD, respectively, in animals treated for 13 wks. There were no unscheduled deaths or drug-related effects on general condition. Mean body wt was reduced in MDM and HDM treated for 4 and 13 wks. In MDM and HDM treated for 4 wks, the body wt effect was observed throughout the dosing period [5-7 and 8-9%, respectively]. In males treated for 13 wks, body wt was affected throughout the dosing period at the HD [5-9%], but only during the first few wks of dosing at the MD [5-6%]. Body wt gain was significantly affected throughout the dosing period in MDM and HDM sacrificed after 4 wks and in HDM sacrificed

after 13 wks; body wt gain was transiently affected [Wks 1-6] in MDM treated for 13 wks. In females treated for 4 wks, mean body wt was not affected; however, body wt gain was significantly reduced [compared to CF] during Wks 2-3 of dosing. In females treated for 13 wks, mean body wt was significantly elevated at the LD and MD [Wks 5/6-13]. Food consumption was significantly reduced throughout the dosing period at all doses in males treated for 4 wks [14-23, 11-28, and 23-30% at LD, MD, and HD, respectively]. In males treated for 13 wks, food consumption was significantly reduced during the first 4 wks of dosing in all dose grps [12-23, 10-28, 17-34% at LD, MD, and HD, respectively], and throughout the dosing period at the HD [8-34%]. In females dosed for 4 and 13 wks, food consumption was reduced [compared to CF] at the MD [14-20%] and HD [13-28%] during the first 3-4 wks of dosing. In females treated for 4 wks, there was an increase in persistent diestrus at all doses [0/15, 6/15, 6/15, and 8/15 CF, LDF, MDF, and HDF, respectively]. In females treated for 13 wks, the incidence of persistent diestrus was increased primarily in MDF and HDF [2/15, 4/15, 9/15, and 10/15 CF, LDF, MDF, and HDF, respectively].

The serum prolactin data are illustrated in the following sponsor's figure and summarized in the following table:

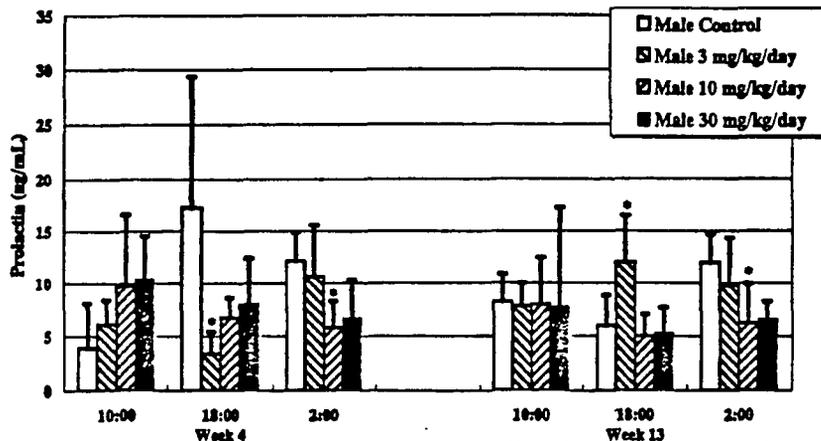


Fig. 1-1 Effect of 13-week Dietary Administration of OPC-14597 on Serum Prolactin Level in ICR Mice
 Item: Serum Prolactin Level Sex: Male
 Each bar represents mean and SD consisted of 5 animals per group.
 Asterisks indicate values which are significantly different from the control : * $p < 0.05$

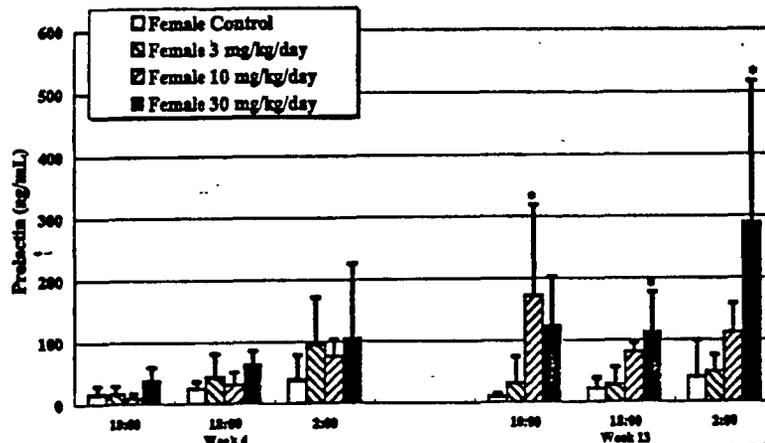


Fig. 1-2 Effect of 13-week Dietary Administration of OPC-14597 on Serum Prolactin Level in ICR Mice
 Item: Serum Prolactin Level Sex: Female
 Each bar represents mean and SD consisted of 3 to 5 animals per group.
 Asterisks indicate values which are significantly different from the control : * $p < 0.05$

WK	DOSE [mg/kg]	MALES			FEMALES		
		10:00	18:00	2:00	10:00	18:00	2:00
4	0	3.96 ± 4.17	17.24 ± 12.11	12.15 ± 2.75	16.28 ± 13.11	24.36 ± 11.47	37.70 ± 40.55
	3	6.16 ± 2.29	3.43 ± 2.00*	10.61 ± 4.98	16.74 ± 12.83	42.25 ± 38.39	95.75 ± 75.64
	10	9.73 ± 6.91	6.76 ± 1.87	5.86 ± 2.46*	10.22 ± 6.41	30.98 ± 20.07	76.35 ± 27.36
	30	10.40 ± 4.18	8.05 ± 4.38	6.71 ± 3.57	38.39 ± 21.25	64.24 ± 21.60	105.78 ± 120.76
13	0	8.26 ± 2.60	6.00 ± 2.83	11.97 ± 2.71	9.50 ± 4.16	21.58 ± 16.68	39.28 ± 60.02
	3	7.85 ± 2.15	12.00 ± 4.47*	9.74 ± 4.60	31.11 ± 42.68	27.72 ± 28.65	48.14 ± 24.74
	10	8.00 ± 4.48	5.01 ± 2.07	6.26 ± 3.68*	172.03 ± 147.22*	80.62 ± 15.30	111.76 ± 47.19
	30	7.80 ± 9.44	5.30 ± 2.40	6.66 ± 1.59	123.61 ± 78.95	113.40 ± 64.09*	289.64 ± 227.30*

*p<0.05

7. Study title: Serum prolactin levels in rats orally given OPC-14597 [Study no: 014065, Conducting laboratory and location: Otsuka Pharmaceuticals Inc., Japan, Date of study initiation: 10/21/97, GLP, QA report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. 97A99M.

Formulation/vehicle: suspension/5% gum arabic in water

Methods

Dosing: OPC-14597 was administered to Sprague-Dawley rats at doses of 0, 1, 3, and 10 mg/kg, either acutely [24/sex/grp] or for 1 wk [6/sex/grp].

Observations and times: body wts were recorded on the day of dosing for animals receiving acute dosing, and on the first day of dosing for those animals treated for 1 wk. Blood samples were collected for quantitation of serum prolactin at 1, 2, 4, and 8 hrs postdosing in acutely treated animals, and at 1 hr postdosing in animals treated for 1 wk. Serum prolactin was quantitated using a rat EIA kit.

Results

In males, serum prolactin was elevated at all doses 1 hr following an acute dose [7.273 ± 5.172, 15.408 ± 0.045, 30.055 ± 11.238, and 31.418 ± 12.423 ng/mL in CM, LDM, MDM, and HDM, respectively; significant at MD and HD], but not 1 hr following 1 wk of treatment. In females, serum prolactin was significantly elevated at the MD and HD following an acute dose, but not following 1 wk of treatment. The data in females are summarized in the following table:

DOSING	SAMPLING TIME [hr]	DOSE [mg/kg]			
		0	1	3	10
acute	1	0.737 ± 0.307	2.888 ± 4.324	63.000 ± 55.914	209.408 ± 77.191**
	2	4.703 ± 8.526	21.017 ± 11.295	105.587 ± 50.481**	199.135 ± 53.896**
	4	34.743 ± 76.787*	4.812 ± 5.614	13.315 ± 6.236	49.247 ± 41.659
	8	4.982 ± 7.150	10.415 ± 8.429	19.547 ± 19.282	39.792 ± 36.224*
7-day	1	89.893 ± 218.924	2.693 ± 2.576	57.142 ± 53.241	96.170 ± 100.140

*p<0.05, **p<0.01, *1 outlier [191.31 ng/mL; range in other animal: 0.30-10.26 ng/mL; mean w/o outlier: 3.43 ng/mL]

8. Study title: Effect of 4-week dietary administration of OPC-31 on serum prolactin level in rat [Study No: 014009, Conducting laboratory and location: Otsuka Pharmaceuticals Inc., Japan, Date of study initiation: 1/14/99, GLP, QA report:Y]

Drug, lot #, radiolabel, and % purity: OPC-031, lot no. 97A99M, purity:

Formulation/vehicle: dietary

Methods

Dosing: OPC-31 was administered as a drug-diet admixture to Fischer 344 rats [10/sex/grp] at oral

doses of 0, 1, 3, and 10 mg/kg for 4 wks.

Observations and times: animals were observed daily for assessment of general condition; mortality/morbidity was assessed twice daily on weekdays. Body wt and food consumption were recorded prior to start of dosing and weekly during dosing. Blood samples were collected [trunk blood] from all survivors at scheduled sacrifice [at 9:00-11:00 a.m.] for assessment of serum prolactin levels. Serum prolactin was quantitated using EIA [rat prolactin]. Blood samples were also collected from 5/sex/grp for analysis of TK; plasma drug levels were quantitated using GC/MS. Following blood sampling, the stage of estrus was determined by examination of vaginal smears and microscopic examination of vagina [10% neutral buffered formalin, H & E].

Results

Examination of the drug-diet admixture confirmed homogeneity and intended drug concentrations. Achieved doses were calculated as 0.955-0.954, 2.87-2.85, 9.47-9.23 mg/kg/day at the LD, MD, and HD, respectively.

There were no drug-related deaths or clinical signs, and no drug-related effects on body wt were observed. Food intake was transiently increased in LDM [6%]; however, overall daily food intake was similar among grps. In females, food intake was reduced [10%] only during the last wk of dosing; overall daily food intake was similar among grps.

Serum prolactin was significantly reduced in HDM [6.37 ± 3.21 , 4.68 ± 2.89 , 5.97 ± 2.14 , and 2.07 ± 0.78 ng/mL for CM, LDM, MDM, and HDM, respectively]. Serum prolactin was not significantly affected in females [4.64 ± 3.68 , 6.33 ± 13.74 , 1.62 ± 1.06 , and 6.06 ± 5.71 ng/mL]; there was also no notable difference among grps in the stage of estrus as assessed by either examination of vaginal tissue or smears. Persistent diestrus was not observed in any female. Plasma levels of OPC-31 were as follows: 0.8 ± 0.2 , 3.0 ± 2.5 , and 15.4 ± 2.6 ng/mL in LDM, MDM, and HDM, respectively, and 2.6 ± 2.6 , 2.7 ± 0.7 , and 18.3 ± 3.8 ng/mL in LDF, MDF, and HDF, respectively.

9. Study title: **Thirteen-week oral investigative study of hormone levels in rats** [Study No: DM00008, Conducting laboratory and location: Bristol-Myers Squibb, Mt. Vernon, Indiana, Date of study initiation: 4/00, GLP, QA report:Y]

Drug, lot #, radiolabel, and % purity: BMS-337039, batch no. C99G74M,
Formulation/vehicle: suspension/5% gum arabic in sterile water

Methods

Dosing: BMS-337039 was administered to Sprague-Dawley rats [20/sex/grp] at doses of 0, 10, 20, 40, and 60 mg/kg [5 mL/kg] p.o. for 13 wks. Ten/sex/grp were sacrificed after 4 wks of dosing [5/sex/grp each at 2 (0900-1000) and 8 (1500-1600) hrs postdosing] and the remaining 10/sex/grp were sacrificed after 13 wks of dosing [5/sex/grp each at 2 and 8 hrs postdosing].

Observations and times: animals were observed for death/morbidity twice daily. Body wts were recorded prior to start of dosing, on Day 1 [pre-dosing], and weekly during the dosing period; data were not reported. Blood samples were collected at sacrifice [trunk blood] for analysis of the following hormones [quantitated by RIA]: serum prolactin, LH, FSH, progesterone [F only], testosterone [M only], estradiol [F, 13-wk only]. Serum prolactin data from animals determined to be in proestrus [n = 4] were excluded from calculation of means. Serum LH data were considered to be unreliable and were not reported. Serum estrogen data from 3 animals [1 C, 2 DT] were removed from summary analyses "...because of inconsistency of duplicate results..."

At 4- and 13-wk necropsy, mammary gland [M, F], ovaries, uterus, vagina, and gross lesions were collected for microscopic analysis. At the 13-wk necropsy only, testes, epididymides, prostate, and seminal vesicles were collected for microscopic analysis. Tissues were preserved in 10% neutral buffered formalin and stained with H & E [4 μ sections] for examination. Special stains []
 "...were used to identify the pigment seen in several ovarian sections".

Results

There was one unscheduled death [HDF #2520]; no cause of death was determined. [The sponsor noted that no drug-related effects were detected in this animal.]

The only significant findings in males were a decrease in serum prolactin at 8 hrs postdosing at the HD [82%] at Wk 4 and an increase in serum testosterone at 8 hrs postdosing at Wk 13 [130%]. However, the following were also noted at Wk 4: (a) a tendency for testosterone to be reduced at doses >10 mg/kg at 8 hr postdosing [59, 64, and 70% at 20, 40, and 60 mg/kg, respectively], (b) tendency for serum prolactin to be elevated at 10 and 20 mg/kg [580 and 120% (i.e., 6.8 and 2.2-fold), respectively] at 2 hrs postdosing and reduced at 40 and 60 mg/kg at 2 and 8 hrs postdosing [2 hr: 68-71%, 8 hr: 78-82%]. FHS was not notably affected. At 13 wks postdosing, the only notable findings in males were: (a) a tendency for serum prolactin to be elevated at 10 mg/kg [250% or 3.5-fold] at 2 hrs postdosing and reduced at 40 and 60 mg/kg [57-64%] at 2 and 8 hrs postdosing. The sponsor considered the decrease in serum prolactin at 40 and 60 mg/kg, although not statistically significant, to be drug-related. The sponsor did not consider testosterone, FSH, or LH to be affected by drug in males. The data were summarized in the following sponsor's tables:

Wk 5

Group	Sex	TEST		FSH		PRL	
		2 HR	8 HR	2 HR	8 HR	2 HR	8 HR
		ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
1m	Mean	1.86	4.58	6.76	8.12	6.92	16.48
	S.D.	1.31	3.21	0.75	2.57	6.30	12.05
	N	5	5	5	5	5	5
2m	Mean	2.62	4.16	8.80	7.64	46.82	9.76
	S.D.	2.62	2.14	6.30	1.28	49.86	10.47
	N	5	5	5	5	5	5
3m	Mean	2.28	1.88	6.88	7.32	15.02	8.78
	S.D.	0.48	0.61	1.65	1.48	20.64	8.02
	N	5	5	5	5	5	5
4m	Mean	1.56	1.63	7.24	7.00	2.18	3.58
	S.D.	1.39	0.85	1.01	0.45	1.18	0.72
	N	5	4	5	5	5	5
5m	Mean	2.10	1.40	5.92	7.12	2.00	2.92*
	S.D.	0.36	1.07	1.15	1.98	0.47	1.53
	N	3	5	5	5	5	5

Wk 13

Group	Sex	TEST		FSH		PRL	
		2 HR	8 HR	2 HR	8 HR	2 HR	8 HR
		ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
1m	Mean	2.52	1.26	6.62	9.10	7.24	10.02
	S.D.	1.30	0.52	2.25	1.42	7.39	6.29
	N	5	5	5	5	5	5
2m	Mean	1.04	1.66	8.74	9.58	25.34	17.82
	S.D.	0.34	0.61	2.13	1.19	22.18	16.96
	N	5	5	5	5	5	5
3m	Mean	1.50	1.66	7.48	8.48	8.14	8.48
	S.D.	0.66	1.21	0.98	1.38	8.62	2.65
	N	5	5	5	5	5	5
4m	Mean	2.18	0.74	9.66	8.50	2.98	3.56
	S.D.	1.41	0.26	1.66	1.10	1.65	1.87
	N	5	5	5	5	5	5
5m	Mean	0.96	2.90**	8.40	9.88	2.90	4.28
	S.D.	0.37	0.86	1.21	1.20	0.82	1.14
	N	5	5	5	5	5	5

In females, the only significant effects were as follows: (a) after 4 wks of dosing, increases in serum prolactin at 10 and 20 mg/kg [3800 and 4880% (i.e., ~39- and 50-fold), respectively] at 2 hrs postdosing, and at 20 mg/kg [700% (i.e., 8-fold)] at 8 hrs postdosing, (b) after 13 wks of dosing, an increase in serum prolactin at 10 mg/kg [1600% (i.e., 17-fold)] at 2 hrs postdosing, and at 40 mg/kg [1700% (i.e., 18-fold)] at 8 hrs postdosing. Serum prolactin tended to be elevated at all doses after both 4- and 13-wks of dosing and at both sampling times [2 and 8 hrs postdosing] [4 wk: 1700 and 1000% (~18- and 11-fold) at 40 and 60 mg/kg, respectively, at 2 hrs and 450 and 650% (5.5- and 7.5-fold) at 40 and 60 mg/kg, respectively, at 8 hrs postdosing; 13 wk: 160 and 330% at 40 and 60 mg/kg, respectively, at 2 hrs and 1700 and 1000% at 40 and 60 mg/kg, respectively, at 8 hrs postdosing]. Serum progesterone tended to be elevated at the lower doses after both 4- and 13-wks postdosing; however, the HD was not affected after either time. Estradiol and FSH were not affected by drug. The data were summarized in the following sponsor's tables:

Wk 5

Group	Sex		PROG 2 HR ng/ml	PROG 8 HR ng/ml	FSH 2 HR ng/ml	FSH 8 HR ng/ml	PRL 2 HR ng/ml	PRL 8 HR ng/ml
1f	Mean		4.06	11.63	5.64	3.08	4.36	8.50
	S.D.		1.22	10.13	1.87	1.98	3.50	6.77
	N		5	4	5	5	5	4
2f	Mean		11.02	32.12	6.36	5.80	170.00**	62.30
	S.D.		11.00	21.03	3.35	8.14	74.57	39.52
	N		5	5	5	5	5	4
3f	Mean		5.88	33.32	3.64	5.92	217.12**	68.22*
	S.D.		5.99	24.17	0.79	2.65	59.53	38.76
	N		5	5	5	5	5	5
4f	Mean		8.30	32.33	3.60	4.05	77.80	46.54
	S.D.		9.59	23.50	1.12	0.66	45.19	30.04
	N		5	3	5	4	5	5
5f	Mean		12.84	10.64	5.00	2.84	46.46	65.04
	S.D.		14.66	8.18	4.11	1.80	56.47	36.12
	N		5	5	5	5	5	5

Wk 13

Group	Sex		ESTR 2 HR pg/ml	ESTR 8 HR pg/ml	PROG 2 HR ng/ml	PROG 8 HR ng/ml	FSH 2 HR ng/ml	FSH 8 HR ng/ml	PRL 2 HR ng/ml	PRL 8 HR ng/ml
1f	Mean		29.8	14.5	7.38	12.94	8.08	7.28	10.76	3.55
	S.D.		33.3	7.4	4.30	4.91	2.71	1.08	19.06	2.30
	N		5	4	5	5	5	5	5	4
2f	Mean		20.8	9.6	18.92	20.18	9.06	8.38	181.76**	13.20
	S.D.		13.9	4.0	17.72	10.78	0.90	1.07	81.79	8.06
	N		4	5	5	5	5	5	5	5
3f	Mean		18.6	19.5	20.32	27.02	7.78	8.48	88.38	50.73
	S.D.		20.3	25.3	15.49	15.12	2.78	1.47	64.11	30.99
	N		5	4	5	5	5	5	5	4
4f	Mean		15.0	8.0	18.40	13.46	7.50	10.46	28.26	64.08*
	S.D.		2.4	5.1	8.47	15.00	1.33	4.77	6.49	44.01
	N		4	5	5	5	5	5	5	5
5f	Mean		11.2	12.4	7.74	7.82	7.80	8.04	46.10	38.40
	S.D.		4.1	3.6	12.47	6.59	1.32	1.08	22.75	25.15
	N		5	5	5	5	5	5	5	5

The sponsor attributed the non-dose-related increase in serum prolactin in females to D₂ antagonist effects at lower doses and D₂ partial agonist effects at the higher doses, or, alternatively, "...drug-related modulation of hypothalamic serotonergic pathways regulating tuberoinfundibular dopaminergic neuronal activity and/or other serotonergic mechanisms of prolactin release..."

There were no apparent drug-related gross findings in ovary, mammary gland, uterus, or vagina after 4 wks of dosing. After 13 wks, the only drug-related gross finding was dark discoloration

in ovary in HDF [0/10, 0/10, 0/10, 0/10, 5/10 at 0, 10, 20, 40, and 60 mg/kg, respectively].
Microscopic findings are summarized in the following table:

TISSUE	FINDING	MALES					FEMALES				
		0	10	20	40	60	0	10	20	40	60
WEEK 4/5											
uterus	atrophy						0/10	2/10	2/10	1/10	2/10
	diestrus						1/10	7/10 ^a	8/10 ^b	6/10	5/10
vagina	diestrus						1/10	7/10 ^a	8/10 ^b	6/10	5/10
	mucification						0/10	8/10 ^b	6/10 ^a	5/10 ^a	2/10
mammary gland	distension w/ secretion	0/10	0/10	0/10	0/10	0/10	0/10	2/10	2/10	4/10	8/10 ^b
	hyperplasia	0/10	0/10	0/10	0/10	0/10	1/10	9/10 ^b	8/10 ^b	9/10 ^b	9/10 ^b
WEEK 13											
ovary	pigment						3/10	3/10	2/10	6/10	7/10
uterus	atrophy						0/10	7/10 ^b	7/10 ^b	5/10 ^a	2/10
	diestrus						3/10	9/10 ^a	8/10	7/10	6/10
	metestrus						5/10	0/10 ^a	1/10	2/10	4/10
vagina	diestrus						3/10	9/10 ^a	8/10	7/10	6/10
	metestrus						5/10	0/10 ^a	1/10	2/10	4/10
	mucification						0/10	8/10 ^b	7/10 ^b	7/10 ^b	2/10
epididymes	number increased [#]	0/10	0/9	0/10	0/10	7/10 ^b					
mammary gland	atrophy	0/10	3/10	2/10	2/10	3/10	0/10	0/10	0/10	0/10	0/10
	distension w/secretion	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	4/10	8/10 ^b
	hyperplasia	0/10	0/10	0/10	0/10	0/10	1/10	8/10 ^b	7/10 ^a	9/10 ^b	9/10 ^b

^ap<0.05, ^bp<0.01, [#]refers to germinal epithelial cells ["various cell types may be represented", e.g., spermatogonia, spermatocytes, spermatids]

Additional information were provided in the following sponsor's summary text tables:

Text Table 1: Incidence of BMS-337039-Related Microscopic Findings in the Ovary, Uterus, Vagina, and Mammary Gland of Interim (Week 5) Necropsy Animals

Dose (mg/kg/day):	0	10	20	40	60
No. of Rats (M/F):	10/10	10/10	10/10	10/10	10/10
Sex:	M/F	M/F	M/F	M/F	M/F
Corpora Lutea (CL), Ovary:					
Mean total CLs/animal	32.2	19.1	17.9	15.8	16.1
Mean percent large-size (functional) CLs	28	53	52	57	40
Estrous Cycle/Change, Vagina/Uterus:					
Diestrus (Persistent)	1	7 (7)	8 (5)	6 (5)	5 (2)
Proestrus, estrus, metestrus	9	3	2	4	5
Mucification, Vaginal Epithelium:					
Minimal/mild severity	-	8	3	4	2
Moderate severity	-	0	3	1	0
Atrophy, Uterus					
Minimal/mild severity	-	2	2	1	2
Hyperplasia, Lobule, Mammary Gland:					
Minimal/mild severity	0/1	0/9	0/8	0/8	0/9
Moderate severity	-	-	-	0/1	-
Distension with Secretion, Mammary Gland:					
Minimal/mild severity	-	0/2	0/2	0/4	0/8
Atrophy, Lobule, Mammary Gland:					
Minimal severity	-	1/0	-	-	-

- Indicates absence of finding in group

Text Table 2: Incidence of BMS-337039-Related Microscopic Findings in Ovary, Vagina, Uterus, Mammary Gland, and Epididymides of End-of-Dose Necropsy (Week 13) Animals

Dose (mg/kg/day):	0	10	20	40	60
No. of Rats (M/F):	10/10	10/10	10/10	10/10	10/10
Sex:	M/F	M/F	M/F	M/F	M/F
Corpora Lutea (CLs), Ovary:					
Mean total CLs/animal	44.2	18.1	24.8	14.2	10
Mean percent large-size (functional) CLs	24	45	44	51	39
Pigment (lipofuscin), Ovary:					
Minimal severity	3	3	2	6	4
Mild severity	-	-	-	-	3
Estrous Cycle/Change, Vagina/Uterus:					
Diestrus (Persistent)	3	9 (8)	8 (6)	7 (7)	6 (2)
Proestrus, estrus, metestrus	7	1	2	3	4
Mucification of Vaginal Epithelium:					
Minimal/mild severity	-	6	5	7	2
Moderate severity	-	2	2	0	0
Atrophy, Uterus					
Minimal/mild severity	-	7	7	5	2
Hyperplasia, Lobule, Mammary Gland:					
Minimal/mild severity	0/1	0/8	0/7	0/7	0/9
Moderate severity	-	-	-	0/2	-
Distension with Secretion, Mammary Gland:					
Minimal/mild severity	-	-	0/1	0/4	0/8
Atrophy, Lobule, Mammary Gland:					
Minimal/mild severity	-	3/0	2/0	2/0	3/0
Number Increased, Spermatogenic Epithelial Cells, Epididymides:					
Minimal severity	-	-	-	-	7

- Indicates absence of finding in group

The sponsor attributed the drug-related effects on the female reproductive system [e.g., corpora lutea, persistent diestrus associated with vaginal mucification and uterine atrophy, mammary gland secretion/hyperplasia] to hyperprolactinemia and associated increases in progesterone. The sponsor pointed out that these findings were seen more prominently at the lower doses, although mammary gland secretion was dose-related.

The sponsor attributed the increased number of spermatogenic epithelial cells [located in epididymal ducts] detected in 7/10 HDM to hyperprolactinemia and noted that the finding was also observed at 60 mg/kg in the male fertility study and the 26-wk toxicity study. The sponsor also noted that similar findings have been observed with haloperidol and reserpine. The sponsor attributed the lack of an effect on spermatogenic epithelial cells [with a similar effect on prolactin] to "...additional mechanisms [that] may be involved or that the hyperprolactinemic response at 60 mg/kg/day persisted longer than at 40 mg/kg/day after each daily dose", but that "An indirect pharmacologic effect...on spermatogenesis via interaction with testicular dopamine receptors..." [e.g., bromocriptine]. The mammary gland atrophy observed in males was attributed to "drug-related hormonal perturbation", as noted with quetiapine.

10. Study title: Effect of 13-week dietary administration of OPC-14597 on serum prolactin level in Fischer rat [Study No: 016482, Conducting laboratory and location: Otsuka Pharmaceuticals Inc., Japan, Date of study initiation: 8/29/00, GLP, QA report:Y]

Drug, lot #, radiolabel, and % purity: OPC-14597, lot no. C98G92(2)M, —

Formulation/vehicle: dietary

Methods

Dosing: OPC-14597 was administered in the diet to Fischer rats [M: 8/grp; F: 13/grp] at doses of 0, 3, 10, and 25 mg/kg for 4 or 13 wks. The methods used were the same as used in the carcinogenicity study conducted using this strain.

Observations and times: animals were observed daily for general condition. Body wt and food consumption were recorded weekly during the dosing period. Blood samples were collected via cannulas implanted into the jugular vein [2-3 days prior to sampling] from 4-7 M/grp and 5-8 F/grp. Serial blood samples were collected from each animal at 10:00, 18:00, and 2:00 on Days 29-30 [4-wk treatment] or on Days 92-93 [13-wk treatment]. Serum prolactin was quantitated using sandwich ELISA [rabbit anti-rat prolactin antibody]. Additional blood samples were collected from 1/sex/grp [treated grps only] for assessment of TK on Day 30 or 93 [3 hrs prior to initiation of light cycle]. Plasma levels of OPC-14597 were quantitated using GC-MS. The estrus cycle was characterized in each animal using vaginal smears collected for 2 wks prior to blood sampling; animals found to be in proestrus were excluded. Following blood sampling on Days 30 and 93, ovaries, uterus, and vagina were collected from all females; tissues were preserved in 10% neutral buffered formalin and stained with H & E for microscopic examination.

Results

Mean achieved doses were calculated to be as follows: 3.41-3.14, 11.09-10.21, and 26.07-23.97 mg/kg/day for 4 wks of treatment, and 2.97-2.93, 9.90-9.74, and 24.10-24.05 mg/kg/day for 13 wks of treatment.

There were no unscheduled deaths and no drug-related clinical signs were observed. Mean body wt was reduced in both HDM and HDF treated for 4 and 13 wks. In animals treated for 4 wks, reduced body wt [relative to Cs] was observed during the first 3 wks of dosing [4-7%]. In those treated for 13 wks, the body wt effect was observed throughout the dosing period; final mean body wts were 16-14% lower in HD animals compared to Cs. Food consumption was also reduced in HDM and HDF treated for 4 and 13 wks. In HD animals treated for 4 wks, reduced food consumption was observed during the last 2-3 wks of dosing [11-17%]. In those treated for 13 wks, reduced food consumption was observed throughout the dosing period [10-24%].

Serum prolactin was significantly reduced in males at all doses following both 4 and 13 wks of treatment. There were no significant effect on serum prolactin levels in females. The data were illustrated in the following sponsor's figure:

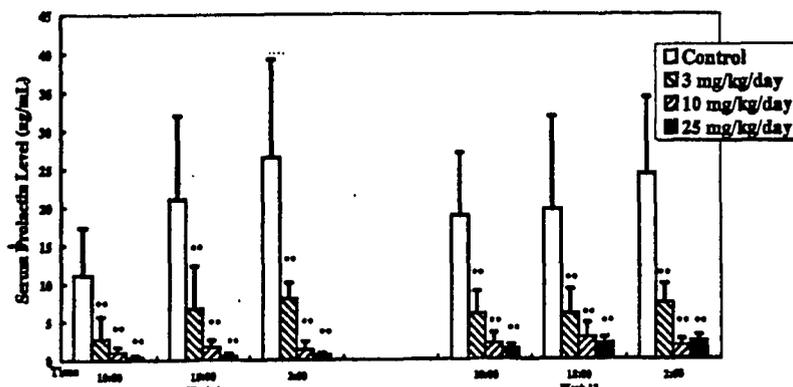


Fig. 1-1 Effect of 13-week Dietary Administration of OPC-14597 on Serum Prolactin Level in Fischer Rats
 Item: Serum Prolactin Level Sex: Male
 Each bar represents mean and SD consisted of 4 to 7 animals per group.
 Asterisks indicates values which are significantly different from the control : **= p<0.01

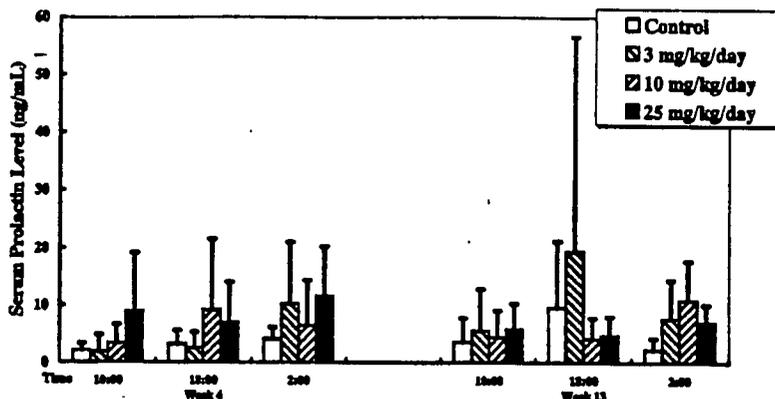


Fig. 1-2 Effect of 13-week Dietary Administration of OPC-14597 on Serum Prolactin Level in Fischer Rats
 Item: Serum Prolactin Level Sex: Female
 Each bar represents mean and SD consisted of 5 to 8 animals per group.
 There were no values which are significantly different from the control.

The data are summarized in the following table [means +/- SD; units: ng/mL]:

DOSE [mg/kg]	WK 4			WK 13		
	10:00	18:00	2:00	10:00	18:00	2:00
MALES						
0	11.2 ± 6.2	21.1 ± 11.0	26.5 ± 12.7	18.9 ± 8.2	19.6 ± 12.2	24.4 ± 9.9
3	2.8 ± 2.9*	6.8 ± 5.5*	8.0 ± 2.1*	6.0 ± 2.9*	6.0 ± 3.2*	7.4 ± 2.5*
10	1.0 ± 0.7*	1.7 ± 1.0*	1.3 ± 1.1*	2.1 ± 1.3*	2.9 ± 1.9*	1.7 ± 0.9*
25	0.3 ± 0.2*	0.5 ± 0.4*	0.6 ± 0.3*	1.4 ± 0.6*	2.0 ± 0.8*	2.4 ± 0.8*
FEMALES						
0	2.1 ± 1.3	3.2 ± 2.4	4.1 ± 2.0	3.6 ± 4.2	9.6 ± 11.6	2.3 ± 2.0
3	1.8 ± 3.0	2.6 ± 2.7	10.2 ± 10.8	5.5 ± 7.3	19.4 ± 37.3	7.6 ± 6.9
10	3.4 ± 3.3	9.1 ± 12.4	6.4 ± 8.0	4.4 ± 4.7	4.1 ± 3.6	10.9 ± 8.6
25	8.9 ± 10.3	6.9 ± 7.2	11.5 ± 8.7	5.8 ± 4.5	4.6 ± 3.4	6.9 ± 3.1

p<0.01

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

Plasma concentrations of OPC-14597 in males and females were as follows.

Sex	Week	Dose (mg/kg/day)		
		3	10	25
Male	4	149	1216	139.77
	13	133	1385	1094.29
Female	4	186	755	347.51
	13	621	1251	282.35

Unit: ng/mL

For comparison, the sponsor also provided a summary of the TK data from the carcinogenicity study in Fischer rat [sponsor's table below]:

Sex	Week	Dose (mg/kg/day)		
		1	3	10
Male	2	0.57	2.33	9.53
	52	0.47	2.07	42.87
	104	0.77	3.03	50.80
Female	2	0.70	2.07	13.73
	52	0.87	4.63	77.47
	104	1.07	5.33	117.53

Unit: ng/mL

Microscopic examination of female reproductive organs indicated an increase in the incidence of persistent diestrus at all doses after both 4 and 13 wks of dosing [4 wk: 0/8 CF, 2/7 LDF, 3/8 MDF, 3/8 HDF; 13 wk: 0/7 CF, 3/8 LDF, 4/7 MDF, and 7/8 HDF].

Although there were significant increases in serum prolactin, the sponsor concluded that a state of hyperprolactinemia was induced in treated animals since (a) "...serum PRL levels at most time points of all treated groups in the 4- and 13-week treated groups were somewhat higher than those of the controls" and (b) "Observations on the stage of estrus cycle indicated high incidence of persistent diestrus in all treated groups..." The sponsor concluded that "Thus, OPC-14597 demonstrated a low potency for increase serum PRL levels in female F344 rats following dietary administration of 3 mg/kg/day or greater." The discrepancy in the serum prolactin effect between males and females was attributed by the sponsor to a partial D₂ agonist effects and to possible activity at "multiple serotonergic receptors".

Adrenal gland

1: **Study title: Thirteen-week oral investigative study in rats** [Study No: DM01013, Conducting laboratory and location: Bristol-Myers Squibb, Mt. Vernon, Indiana, Date of study initiation: 7/10/01, GLP, QA report:Y; 4-wk interim report]

Drug, lot #, radiolabel, and % purity: BMS-337039, batch C00B92M, purity stated as ~~————~~ 'as is'
Formulation/vehicle: suspension/5% gum arabic in sterile water.

Methods

Dosing: BMS-337039 was administered to Sprague-Dawley rats [48/sex/grp] at doses of 0, 10, 20, 40, and 60 mg/kg for 4 wks. At scheduled necropsy, 12/sex/grp were sacrificed 2 hrs postdosing and 12/sex/grp were sacrificed 24 hrs postdosing. The remaining 12/sex/grp were scheduled to be sacrificed following 13 wks of dosing [2 and 24 hrs postdosing]; the data for these animals were not included in this report.

Observations and times: animals were observed for mortality/morbidity twice daily. Body wts were recorded prior to start of dosing and weekly during dosing. [Body wts were not recorded except at necropsy (for calculation of relative organ wt data).]

Blood samples were collected at necropsy [trunk blood] for analysis of plasma ACTH and serum corticosterone. The 2-hr samples were collected at 0900-1100 hrs and the 24-hr samples were collected at 0700-0900 hrs. ACTH and corticosterone were quantitated using RIA. The RIA kit for determination of ACTH was prepared for human plasma; however, the sponsor noted that human assays are valid for analysis of rat ACTH due to the "high molecular homology of rat and human ACTH". The RIA kit for determination of corticosterone was specific for rat.

Adrenal glands were weighed at necropsy. Gross and microscopic examination of adrenal gland was performed in all animals. One adrenal gland per animal was preserved [10% neutral buffered formalin], sectioned, and stained with H & E for examination. The other adrenal gland of each animal was frozen for possible biochemical assays.

Results

There were no unscheduled deaths. Plasma ACTH and serum corticosterone were significantly elevated in HDF [3-fold] at 2 hrs postdosing. Levels at 24 hrs were similar among grps. No effects on either hormone were observed in males. Final mean body wt in males was reduced [compared to CM] at 20 [6%], 40 [11%], and 60 [18%] mg/kg. Body-wt corrected [but not absolute] adrenal wt was significantly increased at 40 [18%] and 60 [30%] mg/kg. Final mean body wt in females was reduced [compared to CF] only at the HD [13%]. Both absolute and

relative adrenal wts were significantly increased in HDF [15-32%]. Upon macroscopic examination, the only finding was an increase in dark discoloration in the adrenal gland in 1/24 and 5/24 females at 40 and 60 mg/kg, respectively. The sponsor proposed that this finding was "possibly secondary to the lipid depletion that may be observed with diffuse adrenocortical hypertrophy". The finding in 1 female at 40 mg/kg was not considered drug-related since it was focal in nature; focal discoloration was not observed in HDF. Upon microscopic examination, an increase in adrenocortical hypertrophy [characterized as diffuse] was observed in HDF [8/24 characterized as minimal and 4/24 characterized as mild; total: 12/24 HDF]. The adrenocortical hypertrophy was said to include both the zona reticularis and zona fasciculata in all affected animals. The sponsor attributed the adrenal gland effect to the 5HT_{1A} partial agonist properties of BMS-337039.

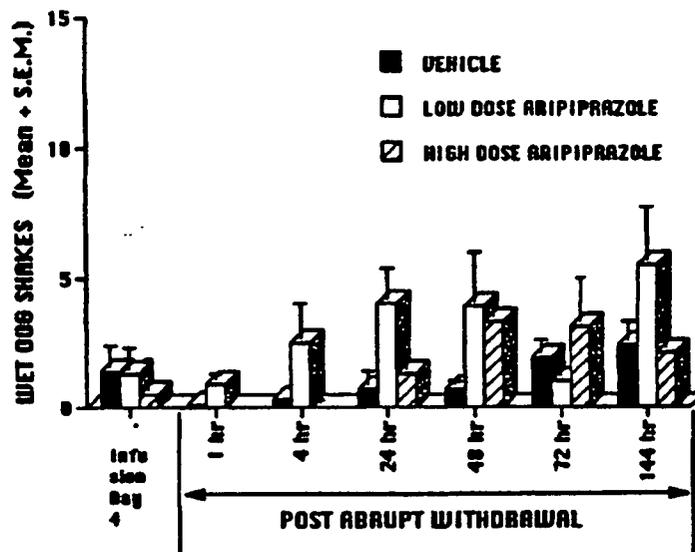
Dependence

Four studies were conducted [2 in male Sprague-Dawley rat, 2 in rhesus monkey] in order to assess the abuse potential of aripiprazole.

Rat: 5 male Sprague-Dawley rats trained to self-administer cocaine [0.3 mg/kg] did not continue to self-administer when aripiprazole [0.1-1 mg/kg i.v.] was substituted for cocaine.

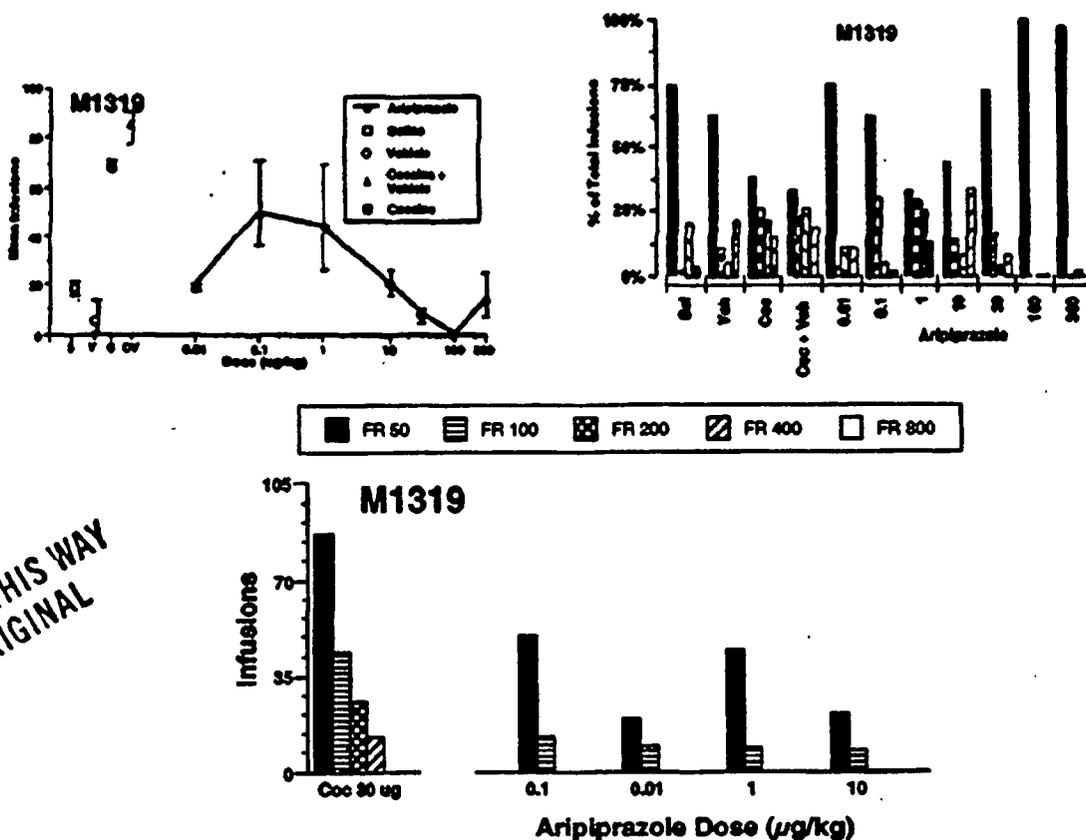
Physical dependency was assessed in male rats [8/grp] using a low-dose [0.75-6 mg/kg i.p.] and a high-dose [12-96 mg/kg i.p.] regimen. Aripiprazole was administered continuously over 4 days. An additional group of 7 males received vehicle [i.p.] over 4 days. At the end of the 4 days treatment period, aripiprazole infusions were stopped and all animals received infusions of vehicle for an additional 5 days. Signs of withdrawal [i.e., wet-dog shakes, facial rubs, and irritability] were recorded during the 5-day "withdrawal" period. The sponsor stated that there were no significant increases in withdrawal signs and no stereotypes were observed in aripiprazole-treated animals. However, only data for wet-dog shakes and facial rubs were provided, and only in figure form. The incidences of wet-dog shakes were illustrated in the following sponsor's figure:

Figure 2



The sponsor noted that "During withdrawal, two signs designated lateral extension and retraction of limbs and standing were noted in some rats; however, their frequency lacked statistical support". It was noted that 3 animals were affected and that "The incidence of this sign ['front- and hind-limb episodes'] was greatest 1- and 4-hr post withdrawal"; however, what grp(s) the affected animals belonged to was not specified. The report of this study did not provide enough data for an adequate review.

Monkey: self-administration of aripiprazole [0.01-300 µg/kg i.v.] was tested in 4 rhesus monkeys trained to self-administer cocaine [30 µg/kg i.v.]. If an animal self-administered aripiprazole, a progressive ratio schedule was instituted in order to quantify the magnitude of the reinforcing effect. Self-administration of aripiprazole was observed in 1/4 monkeys [#1319]. The dose-dependency of this effect was an inverted U-shaped function. The data for the affected animal were illustrated in the following sponsor's figures:



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The sponsor noted that the "break point" for self-administration of aripiprazole in #1319 was FR100, less than the break point observed for cocaine [i.e., FR400]. [The break point was defined as that ratio resulting in a number of self-administrations similar to vehicle.] In addition, the sponsor noted that for cocaine, animals tended to self-administer throughout the testing period, whereas for vehicle, responses were greatest during the first portion of each session. In #1319, the pattern of self-administration of aripiprazole was more like that of vehicle than of cocaine. The sponsor concluded that the data "...suggested marginal abuse liability for aripiprazole".

The potential of aripiprazole to induce physical dependency was tested in 4 rhesus monkeys [1 M, 3 F]. Aripiprazole was administered s.c. 4-6 times per day for 44 days according to the following schedule: Day 1: 0.75 mg/kg/day, Day 2: 2 mg/kg/day, Days 3-8: 1 mg/kg/day, Days 9-14: 2 mg/kg/day, Days 15-22: 3 mg/kg/day, Days 23-28: 5 mg/kg/day, Day 29: 6.25 mg/kg/day [last injection at 6:00 p.m.], Day 30: 1.25 mg/kg/day [first injection at 2:00 p.m.], Days 31-33: 7.5 mg/kg/day, Days 34-35: 5 mg/kg/day, Days

36-40: 7.5 mg/kg/day, Days 41-42: 5 mg/kg/day, Day 43: 7.5 mg/kg/day, Day 44: 1.25 mg/kg/day, Day 45: no dose. Withdrawal signs [i.e., "pacing; wet-dog shakes; vocalizing; fighting; yawning, ...nail biting) and scratching". were evaluated on Days 29 and 44, 15-16 hrs following last daily dose. The data were summarized in the following sponsor's tables:

Table 4. Abrupt withdrawal of aripiprazole: forty-five day evaluation.

Table 3. Abrupt withdrawal of aripiprazole: thirty-day evaluation.

Withdrawal Sign	Number of monkeys showing sign/ Total number		
	Observation Period (min)		
	0 - 30	31 - 60	61 - 90
Nail biting	3/4	2/4	0/4
Pacing	3/4	3/4	3/4
Wet-dog shakes	3/4	1/4	0/4
Vocalizing	1/4	1/4	2/4
Fighting	1/4	1/4	1/4
Yawning	1/4	0/4	0/4
Rigid abdominal muscles	0/4	0/4	0/4

Withdrawal Sign	Number of monkeys showing sign/Total number			
	Observation Period (min)			
	0 - 30	31 - 60	61 - 90	91 - 120
Nail biting	4/4	2/4	2/4	1/4
Lying down	0/4	0/4	1/4	1/4
Brow	1/4	2/4	2/4	1/4
Preening				
Pacing	4/4	4/4	4/4	4/4
Wet-dog shakes	1/4	3/4	1/4	1/4
Vocalizing	1/4	2/4	0/4	0/4
Fighting	0/4	2/4	1/4	0/4
Yawning	1/4	2/4	0/4	0/4
Scratching	3/4	3/4	4/4	2/4
Rigid abdominal muscles	0/4	0/4	0/4	0/4

The sponsor noted that nail biting was a unique sign, "...not...expressed as a withdrawal sign with other classes of drugs. Its significance is unknown." The sponsor concluded that "...physical dependence on aripiprazole, expressed as a mild arousal syndrome, was observed. The syndrome was not associated with distress....and appeared to be limited and benign."

Metabolites

The acute toxicity of two major circulating metabolites, OPC-14857 and OPC-3733, was assessed in rats. OPC-14857 was tested at doses of 50-200 mg/kg i.v. [doses of 50-150 mg/kg were used in the definitive study; n = 5-7/sex/grp]. All animals treated at doses of 150 and 200 mg/kg died. One F died following a 100-mg/kg dose. Drug-related clinical signs [e.g., reduced spontaneous motor activity, "creeping position", hypothermia] were observed at 50 and 100 mg/kg. Microscopic findings were detected in kidney of all animals that died [100-150 mg/kg; histopathology not performed for the 200-mg/kg dose]. Findings consisted of eosinophilic material in Bowman's space and in lumen of cortical proximal tubules [all animals that died], and unilateral papillary necrosis in 1 MDF survivor. The renal effects were considered secondary to hemolysis. Evidence of hemolysis was observed in satellite animals dosed for assessment of TK. Plasma levels of OPC-14857 were as follows:

50 mg/kg: $C_{5 \text{ min}}$ = 7.5 and 4.7 $\mu\text{g/mL}$ in males and females, respectively. $AUC_{(0-8 \text{ hr})}$ = 13.5 and 9.9 $\mu\text{g}\cdot\text{hr/mL}$ in males and females, respectively.

100 mg/kg: $C_{5 \text{ min}}$ = 16.1 and 13.2 $\mu\text{g/mL}$ in males and females, respectively. $AUC_{(0-8 \text{ hr})}$ = 41.5 and 29.8 $\mu\text{g}\cdot\text{hr/mL}$ in males and females, respectively.

The acute toxicity of OPC-3373 was tested in Sprague-Dawley rats [5/sex/grp] at a dose of 1000 mg/kg i.v. [C grps included]. Additional animals [22-23/sex] were dosed in order to obtain TK data. No effects were observed on any of the parameters assessed [i.e., clinical signs, body wt, food consumption, gross pathology]. Plasma levels of OPC-3373 were as follows:

$C_{5 \text{ min}}$: 2531 and 1680 $\mu\text{g/mL}$ in males and females, respectively.
 $AUC_{(0-8 \text{ hr})}$: 1965 and 1121 $\mu\text{g}\cdot\text{hr/mL}$ in males and females, respectively.

The acute toxicity of OPC-14857 and OPC-3373 administered s.c. was tested in Sprague-Dawley rats [2/sex/grp] at doses of 100 and 1000 mg/kg, respectively. [C grps were included.] The only drug-related finding was reddish urine in all animals treated with OPC-14857. No gross findings were observed.

Degradants

Studies were conducted in order to qualify degradants. According to the review chemist [Sherita McLamore, Ph.D.], these degradants are not present in the to-be-marketed drug product at levels requiring qualification. Therefore, these studies were not reviewed for this application.

Special Toxicology Summary and Conclusions

Special toxicology studies were conducted in order to assess the antigenicity/immunotoxicity of aripiprazole and the effects of aripiprazole on serum prolactin and on adrenal gland, the abuse potential, and the acute i.v. toxicity of selected metabolites.

Antigenicity/immunotoxicity: aripiprazole was negative in the passive cutaneous and active systemic anaphylaxis assays in male Hartley guinea pigs. Following 4 wks of dosing, aripiprazole did not affect the antibody response to sheep rbc's in Sprague-Dawley male rats, but did significantly increase the response in females. The response in females was not dose-related.

Serum prolactin: aripiprazole's effect on serum prolactin was not quantitated in the definitive toxicology or carcinogenicity studies. Therefore, the sponsor conducted a number of special studies to assess the effects of oral [gavage, dietary] aripiprazole on serum prolactin levels in ICR [CD-1] mouse and Sprague-Dawley and Fischer 344 rat.

In mouse, serum prolactin was markedly elevated in females following acute oral doses of 3, 10, and 30 mg/kg. Highest levels occurred at 1 hr postdosing [\approx 500 times control value]; however, serum prolactin was still elevated at the last time point analyzed [4 hrs postdosing, \approx 150-200 fold]. Increases in serum prolactin were not dose-related; the magnitude of the effect was similar at all doses at all sampling times. Following dietary administration of aripiprazole for 1 wk at a dose of 30 mg/kg, serum prolactin was not significantly affected. Haloperidol, tested at a dose the sponsor noted was associated with pituitary tumors [i.e., 5 mg/kg, dietary], also failed to significantly elevate serum prolactin. However, there was a tendency for serum prolactin to be higher than control [\approx 9 and 8-fold for aripiprazole and haloperidol, respectively], and the incidence of persistent diestrus was increased in both treated grps. Following dietary administration of aripiprazole at doses of 3, 10, and 30 mg/kg for 4-wks, serum prolactin was

significant reduced at 30 mg/kg in males [42%] and tended to be lower than control at the lower doses [25-27%, not statistically significant]. Serum prolactin was not affected in females; however, persistent diestrus was observed only in treated females. Following dietary administration of aripiprazole for 13 wks at doses of 3, 10, and 30 mg/kg, there was no consistent effect on serum prolactin in males. At Wk 4, the only significant effects in males were decreases in serum prolactin at 3 and 10 mg/kg [80 and 52%, respectively]. There were no significant effects at 30 mg/kg; however, serum prolactin tended to be both higher and lower than control depending on the sampling time. At Wk 13, serum prolactin was significantly elevated in males at 3 mg/kg [100%] and significant decreased [48%] at 10 mg/kg. In females, serum prolactin was not significantly affected at Wk 4. At Wk 13, serum prolactin was significantly increased at 10 and 30 mg/kg [18- and 5-13 fold, respectively].

In Sprague-Dawley rats, an acute 20-mg/kg oral dose markedly elevated serum prolactin [25-fold] in females. In a 1-wk gavage study [1, 3, 10 mg/kg], serum prolactin was elevated in males and females at all doses following an acute dose [M; 2-, 4-, and 4-fold, respectively; F: 4-, 20-, and 200-fold, respectively], although the effect at 1 mg/kg was not statistically significant in either males or females. After 1 wk of dosing, serum prolactin was not significantly affected in either males or females. In a 13-wk "investigative" study, aripiprazole was administered by gavage at doses of 10, 20, 40, and 60 mg/kg. The only significant effects observed in males were increases in serum prolactin [Wk 4] and in serum testosterone [Wk 13]. However, serum prolactin tended to be elevated at 10 and 20 mg/kg, and decreased at 40 and 60 mg/kg during Wks 4 and 13. [FSH was not affected.] In females, serum prolactin was significantly increased at 10 and 20 mg/kg after 4 wks of dosing, and at 10 and 40 mg/kg after 13 wks of dosing. However, in females, serum prolactin tended to be elevated at all doses at Wks 4 and 13; however, the effect was greater at the lower doses. Serum progesterone also tended to be higher at the lower doses. Upon microscopic examination of male and female reproductive organs, the only finding in males was an increase in germinal epithelial cells in the epididymes at 60 mg/kg. [The sponsor attributed this effect to increases in serum prolactin. In females, the number of animals in diestrus was increased in all dose grps; however, the effect was not dose-related. The incidences of "diestrus" in uterus and vagina were significantly increased only at the lower dose grps, not 60 mg/kg. The incidence of uterine atrophy was significantly increased at doses of 10-40 mg/kg, but not at 60 mg/kg [Wk 13]. Mammary gland hyperplasia was increased to a similar extent at all doses; distension with secretion was increased in a dose-related manner [significant only at 60 mg/kg].

In a 4-wk study in Fischer 344 rats, aripiprazole was administered in the diet at doses of 1, 3, and 10 mg/kg. Serum prolactin was significantly reduced in males at 10 mg/kg [68%], but was not significantly affected in females. There was also no notable effect on the stage of estrus as assessed either by microscopic examination of vaginal tissue or by vaginal smear; persistent diestrus was not observed in any female. In a 13-wk dietary study, aripiprazole was administered at doses of 3, 10 and 25 mg/kg. Serum prolactin was significantly reduced in males at all doses at both Wk 4 and Wk 13. Serum prolactin was not significantly affected in females during either wk. A comparison of the TK data from the special study and the 2-yr carcinogenicity study indicated that Wk 4 plasma levels of aripiprazole were similar for the 3-mg/kg dose grps. At 10 mg/kg, steady-state plasma levels achieved in the 2-yr study were 3-4 and 6-9 times higher than those achieved in the special toxicity study [Wk 13 data] in males and females, respectively. However, plasma levels of aripiprazole achieved at 25 mg/kg in the special toxicity study were \approx 20 and 2.5 times higher than those achieved in males and females, respectively, in the 2-yr study at 10 mg/kg [the high-dose]. Microscopic examination of female reproductive organs indicated an increase in the incidence of persistent diestrus at all doses in the special toxicity study.

Adrenal gland: the sponsor initiated a 13-wk study in Sprague-Dawley rats in order to further investigate the effects of aripiprazole on adrenal gland. A 4-wk interim report was submitted for this study. [No 13-wk study report could be found.] Circulating levels of ACTH and corticosterone were quantitated, and adrenal glands were microscopically examined following 4 wks of [gavage] dosing at 10, 20, 40, and 60

mg/kg [n = 12/sex/grp]. ACTH and corticosterone were significantly elevated in females at 60 mg/kg [2 hrs postdosing]. No hormone effects were observed in males. The data were summarized in the following sponsor's tables:

Week: 5 relative to Start Date					Week: 5 relative to Start Date						
Group Sex		2 Hour ACTH pg/ml	24 Hour ACTH pg/ml	2 Hour Corticos ng/ml	24 Hour Corticos ng/ml	Group Sex		2 Hour ACTH pg/ml	24 Hour ACTH pg/ml	2 Hour Corticos ng/ml	24 Hour Corticos ng/ml
1m	Mean	19.03	18.28	27.9	21.1	1f	Mean	13.82	21.69	55.7	95.8
	S.D.	10.95	9.27	30.4	17.5		S.D.	9.02	10.27	66.1	60.0
	N	12	12	12	12		N	12	12	12	12
2m	Mean	19.27	13.82	18.7	13.4	2f	Mean	12.18	22.91	33.6	68.3
	S.D.	12.36	5.42	10.2	10.4		S.D.	5.20	10.57	11.0	46.1
	N	12	12	12	12		N	12	12	12	12
3m	Mean	19.01	17.77	9.3	27.1	3f	Mean	8.24	17.37	32.8	54.6
	S.D.	29.71	13.00	4.4	18.0		S.D.	5.66	8.69	17.5	17.5
	N	12	12	12	12		N	12	12	12	12
4m	Mean	22.35	25.03	22.3	52.3	4f	Mean	10.66	17.47	46.3	89.3
	S.D.	27.39	18.07	24.7	77.6		S.D.	9.40	8.85	60.2	59.2
	N	12	12	12	12		N	12	12	12	12
5m	Mean	26.50	20.30	54.2	48.3	5f	Mean	42.26**	19.27	159.3**	99.0
	S.D.	37.45	21.98	77.3	42.8		S.D.	45.11	7.42	124.1	62.5
	N	12	12	12	12		N	12	12	12	12

Adrenal wt was increased in females at 60 mg/kg [15-32%]. Upon microscopic examination, adrenocortical hypertrophy [characterized as diffuse, with minimal-to-mild severity] was observed in females at 60 mg/kg. Both the zona fasciculata and zona reticularis were involved in all affected females. No dose-related microscopic findings were detected in males. The sponsor attributed the adrenal gland effects to the 5HT_{1A} partial agonist activity of aripiprazole.

Dependence: four studies were conducted, 2 in male Sprague-Dawley rat and 2 in rhesus monkey, in order to assess the abuse potential of aripiprazole.

Male rats trained to self-administer cocaine did not continue to self-administer when aripiprazole (i.v.) was substituted for cocaine. In a physical dependency study, animals were observed for 5 days after four days of treatment with aripiprazole [administered at as a low-dose or high-dose regimen]. According to the sponsor, there were no significant increases in withdrawal symptoms such as wet-dog shakes or facial rubs. However, data were provided only for one symptom [wet-dog shakes] and only in figure form. From the figure, it appeared that there was a tendency for the incidence of wet-dog shakes to be higher in animals receiving the low-dose regimen. The sponsor also noted that 3 animals exhibited "lateral extension and retraction of limbs" and standing, signs that were not significantly increased, but that were more frequently observed at 1 and 4 hrs post withdrawal. What dose(s) the affected animals received was not stated. The report did not provide sufficient data to allow for an adequate review.

Of 4 rhesus monkeys trained to self-administer cocaine, one continued to self-administer when aripiprazole (i.v.) was substituted for cocaine. The rate of self-administration exhibited an inverted U-shape function with increasing dose. The highest rates were obtained at doses of 0.1 and 1 µg/kg i.v., with rates lower at doses of 0.01, 100, and 300 µg/kg. The pattern of responding within sessions for aripiprazole was noted to be different than for cocaine. In animals self-administering cocaine, a fairly steady rate of responding was obtained throughout the session; with aripiprazole, the greatest rate of responding was early in the session. When the strength of the self-administration response was tested in this monkey, the "break point" [i.e., the FR ratio at which the rate of response was similar to control] was reached at FR 100. For comparison, the break point for cocaine was FR400. According to the sponsor, these data "...suggested a marginal abuse liability for aripiprazole". In a physical dependency study,

aripiprazole [0.75-7.5 mg/kg s.c.] was administered to 4 rhesus monkeys for 44 days. On Days 29-30, dosing was withheld for some period to assess withdrawal symptoms; dosing was stopped following treatment on Day 44. After temporary cessation of dosing [Day 30], withdrawal signs [primarily wet-dog shakes, pacing] were each observed in 3/4 monkeys; pacing continued to be observed throughout the 90-min assessment period. On Day 45, these signs were observed in all animals as well as scratching [3/4 monkeys]; yawning, vocalizations, and fighting were each observed in 1-2 of 4 monkeys. Withdrawal signs continued to be observed throughout the 120-min observation period. Nail biting was observed in 3/4 monkeys on Day 30 and all monkeys on Day 45. According to the sponsor, nail biting was a unique behavior not observed with other classes of drug that induce dependency. Based on these data, the sponsor concluded that aripiprazole induced physical dependence, characterized as a "mild arousal syndrome", and noted that the syndrome was not associated with distress and "appeared to be limited and benign".

From these data, it would appear that aripiprazole may have some abuse potential. The abuse/physical dependency studies have not been reviewed by the Controlled Substances Staff. However, the data were discussed with a member of that staff, Sylvia Calderon, Ph.D. It was Dr. Calderon's opinion that the data suggest the possibility of some abuse potential for aripiprazole, and that this potential should be further investigated. It was also Dr. Calderon's opinion that a statement on abuse potential should be included in labeling. The studies on aripiprazole have been consulted to the Controlled Substances Staff for consultative review, and on the basis of that review, additional guidance may be submitted to the sponsor.

Acute toxicity of metabolites, OPC-14857 and OPC-3373: the acute toxicity of two major circulating metabolites was tested in Sprague-Dawley rats. OPC-14857 induced 100% lethality at doses of 150 and 200 mg/kg i.v.; one animal died at 100 mg/kg. Drug-related clinical signs [i.e., decreased spontaneous motor activity, "creeping position", hypothermia] were evident at all doses. Microscopic findings were detected in kidney in all animals that died; findings consisted of eosinophilic material in Bowman's space and in lumen of the cortical proximal tubules. Unilateral papillary necrosis was detected in 1 survivor at 100 mg/kg. The renal effects were attributed to hemolysis, which was stated to be evident in blood samples collected for analysis of TK. Following acute s.c. doses of OPC-14857 [100, 1000 mg/kg], the only finding was reddish urine in all rats; this finding is consistent with hemolysis.

OPC-3373 had no effect on any parameter assessed, including clinical signs, when administered acutely at a dose of 1000 mg/kg i.v. or at doses of 100 and 1000 mg/kg s.c.

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IX. RECOMMENDATIONS

From a pharmacology/toxicology standpoint, the data submitted for aripiprazole support approval of the NDA. However, there are two issues that the sponsor would need to address postmarketing:

(a) additional studies should be conducted in order to further characterize and, if possible, to determine the mechanism(s) underlying the retinal degeneration observed in the 26-wk and 2-yr carcinogenicity studies in Sprague-Dawley rat.

(b) the data from studies conducted in rhesus monkey suggest that aripiprazole may have some abuse liability. One of 4 monkeys trained to self-administer cocaine continued to self-administer when aripiprazole was substituted for cocaine. In addition, 4 of 4 monkeys exhibited withdrawal symptoms following abrupt cessation of dosing with aripiprazole. Although self-stimulation was not observed in rats when aripiprazole was substituted for cocaine, there was a tendency for animals to exhibit withdrawal symptoms following abrupt cessation of dosing. Therefore, additional studies investigating the abuse liability of aripiprazole should be conducted.

Labeling Recommendations

CLINICAL PHARMACOLOGY

Pharmacodynamics

Aripiprazole exhibited high affinity for dopamine D₂ and D₃, serotonin 5-HT_{1A} and 5-HT_{2A} receptors (K_i values of 0.34, 0.8, 1.7 and 3.4 nM, respectively); and moderate affinity for dopamine D₄, serotonin 5-HT_{2C} and 5-HT₇, alpha₁-adrenergic and histamine H₁ receptors (K_i values of 44, 15, 39, 57, and 61 nM respectively) and the serotonin reuptake site (K_i = 98 nM). Aripiprazole had no appreciable affinity for cholinergic muscarinic receptors (IC₅₀ > 1000 nM). Aripiprazole functioned as a partial agonist at the dopamine D₂ and the serotonin 5-HT_{1A} receptors, and as an antagonist at serotonin 5-HT_{2A} receptor.

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

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Lifetime carcinogenicity studies were conducted in ICR mice and in Sprague-Dawley (SD) and F344 rats. Aripiprazole was administered for two years in the diet at doses of 1, 3, 10, and 30 mg/kg/day to ICR mice and 1, 3, and 10 mg/kg/day to F344 rats (0.2 to 5 and 0.3 to 3 times the maximum recommended human dose [MRHD] on a mg/m² basis, respectively). In addition, SD rats were dosed orally for two years at 10, 20, 40, and 60 mg/kg/day (3 to 19 times the MRHD on a mg/m² basis). Aripiprazole did not induce tumors in male mice or rats. In female mice, the incidences of pituitary gland adenomas and mammary gland adenocarcinomas and adenoacanthomas were increased at dietary doses of 3 to 30 mg/kg/day (0.5 to 5 times the MRHD on a mg/m² basis). In female rats, the incidence of

mammary gland fibroadenomas was increased at a dietary dose of 10 mg/kg/day (3 times the MRHD on a mg/m² basis); and the incidences of adrenocortical carcinomas and combined adrenocortical adenomas/carcinomas were increased at an oral dose of 60 mg/kg/day (19 times the MRHD on a mg/m² basis).

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

Mutagenesis

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

Impairment of fertility

Female rats were treated with oral doses of 2, 6, and 20 mg/kg/day (0.6, 2, and 6 times the maximum recommended human dose [MRHD] on a mg/m² basis) of aripiprazole from 2 weeks prior to mating through day 7 of gestation. Estrus cycle irregularities and increased corpora lutea were seen at all doses, but no impairment of fertility was seen. Increased pre-implantation loss was seen at 6 and 20 mg/kg, and decreased fetal weight was seen at 20 mg/kg.

Male rats were treated with oral doses of 20, 40, and 60 mg/kg/day (6, 13, and 19 times the MRHD on a mg/m² basis) of aripiprazole from 9 weeks prior to mating through mating. Disturbances in spermatogenesis were seen at 60 mg/kg, and prostate atrophy was seen at 40 and 60 mg/kg, but no impairment of fertility was seen.

PREGNANCY

Pregnancy Category C

(Note to sponsor: doses causing fused vertebrae in rabbits may be changed pending submission of historical control data)

In animal studies aripiprazole demonstrated developmental toxicity, including possible teratogenic effects in rat and rabbit.

Pregnant rats were treated with oral doses of 3, 10 and 30 mg/kg/day (1, 3, and 10 times the maximum recommended human dose [MRHD] on a mg/m² basis) of aripiprazole during the period of organogenesis. Gestation was slightly prolonged at 30 mg/kg. Treatment caused a slight delay in fetal development as evidenced by decreased fetal weight (30 mg/kg), undescended testes (30 mg/kg), and delayed skeletal ossification (10 and 30 mg/kg). There were no adverse effects on embryofetal or pup survival. Delivered offspring had decreased bodyweights (10 and 30 mg/kg), and increased incidences of hepatodiaphragmatic nodules (liver protrusion through the diaphragm) and diaphragmatic hernia (30 mg/kg; the other dose groups were not examined for these findings). (A low incidence of diaphragmatic hernia was also seen in the fetuses exposed to 30 mg/kg.) Postnatally, delayed vaginal opening was seen at 10 and 30 mg/kg and impaired reproductive performance (decreased fertility rate, corpora lutea, implants, and live fetuses, and increased post-implantation loss, likely mediated through effects on female offspring) was seen at 30 mg/kg. Slight maternal toxicity was seen at 10 and 30 mg/kg; however, there was no evidence to suggest that these developmental effects were secondary to maternal toxicity.

Pregnant rabbits were treated with oral doses of 10, 30, and 100 mg/kg/day (6, 19, and 65 times the MRHD on a mg/m² basis) of aripiprazole during the period of organogenesis. Decreased maternal food consumption and increased abortions were seen at 100 mg/kg. Treatment caused increased fetal mortality (100 mg/kg), decreased fetal weight (30 and 100 mg/kg), and an increased incidence of skeletal abnormalities (fused sternbrae) and minor skeletal variations (100 mg/kg).

In a study in which rats were treated with oral doses of 3, 10, and 30 mg/kg/day (1, 3, and 10 times the MRHD on a mg/m² basis) of aripiprazole peri- and post-natally (from day 17 of gestation through day 21 post-partum), slight maternal toxicity and slightly prolonged gestation were seen at 30 mg/kg. An increase in stillbirths, and decreases in pup weight (persisting into adulthood) and survival, were seen at this dose.

ANIMAL TOXICOLOGY

Aripiprazole produced retinal degeneration in rats in a 26-wk chronic toxicity study at a dose of 60 mg/kg and in a 2-yr carcinogenicity study at doses of 40 and 60 mg/kg. The 40- and 60-mg/kg doses are 13 and 19 times the maximum recommended human dose on a mg/m² basis. The relevance of this finding to human risk is unknown.

X. APPENDIX/ATTACHMENTS

Addendum to review: Exe-CAC minutes for the meeting [July 16, 2002] on the carcinogenicity studies for aripiprazole.

**APPEARS THIS WAY
ON ORIGINAL**

EXECUTIVE CAC**Date of Meeting: 7/16/02****Mouse/Rat Carcinogenicity Studies**

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Jim Farrelly Ph.D., HFD-530, Alternate Member
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Barry Rosloff, Ph.D., Supervisory Pharmacologist
Lois Freed, Ph.D. HFD-120, Presenting Reviewer

Author of Draft: Lois M. Freed, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA #21-436**Drug Name:** aripiprazole**SPONSOR:** OTSUKA PHARMACEUTICALS

Mouse Carcinogenicity Studies: two 2-yr dietary carcinogenicity studies were conducted in CD-1 mice. Study 1 was conducted at doses of 0, 1, 3, and 10 mg/kg. No dose-limiting toxicities were observed. No drug-related tumors were detected in male mice. In female mice, there were significant increases in anterior pituitary adenomas and mammary gland tumors [adenocarcinoma, adenoacanthoma] at 3 and 10 mg/kg. Study 2 was conducted at doses of 0 and 30 mg/kg. Body weight was reduced in drug-treated males [10%] relative to control males. Mortality rate was significantly increased in drug-treated females. No drug-related tumor findings were detected in male mice. In female mice, there were significant increases in anterior pituitary adenoma and mammary gland tumors [adenocarcinoma, adenoacanthoma]. The sponsor attributed the neoplastic findings to increases in serum prolactin [not measured in the carcinogenicity studies]; a direct drug-effect on DNA synthesis in the pituitary was also suggested as a possible mechanism underlying the increase in pituitary adenomas.

Rat Carcinogenicity Studies: two 2-yr oral carcinogenicity studies were conducted in rats. Study 1 was conducted in Fischer 344 rats at doses of 0, 1, 3, and 10 mg/kg. No dose-limiting toxicities were observed. No drug-related tumors were detected in male rats. In female rats, there was a significant increase in mammary gland fibroadenomas at the HD. Study 2 was conducted in Sprague-Dawley rats at doses of 0, 0, 10, 20, 40, and 60 mg/kg. Dose-related decreases in body weight [compared to controls] were observed in both males and females. No drug-related tumor findings were detected in male rats. In female rats, there was a significant increase in adrenocortical tumors [carcinoma, combined adenoma and carcinoma]. No mechanism was proposed by the sponsor for the adrenocortical tumors.

Executive CAC Recommendations and Conclusions: the ExeCAC concluded that the assessment of carcinogenic potential was adequate in both mice and rats based on body wt effects in male mice, male rats, and female rats and on an increase in mortality in female mice at the highest doses tested. Aripiprazole was negative for neoplasms in male mice and rats. In female mice, pituitary adenomas and mammary gland tumors [adenocarcinoma, adenoacanthoma] at 3, 10, and 30 mg/kg were considered drug-related. In female rats, the increase in mammary gland fibroadenomas at 10 mg/kg in Study 1 and the increase in adrenocortical tumors [carcinoma, combined adenoma/carcinoma] at 60 mg/kg in Study 2 were considered drug-related.

The Committee recommended that

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\

/Division File, HFD-120
/BNRosloff, HFD-120
/LMFreed, HFD-120
/SHardeman, HFD-120
/ASeifried, HFD-024

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PHARMACOLOGIST REVIEW OF GLP EIR
(CP 7348.808)

FIRM NAME: Bristol-Myers Squibb Company
CITY, STATE: Mt. Vernon, IN

QUARTER/FY ASSIGNED: 2/01
EI DATE(S): 8/6-13/01

FEI: 1831144
DISTRICT OFFICE: Detroit, HFR-CE750

INVESTIGATOR(S): 1. Larry K. Austin, EVS-RP, HFR-CE7545
2.
3.

INSPECTION TYPE:	<input checked="" type="checkbox"/>	ROUTINE SURV.	<input type="checkbox"/>	DIRECTED
FDA-483 ISSUED:	<input checked="" type="checkbox"/>	NO	<input type="checkbox"/>	YES
LETTER TO ISSUE:	<input checked="" type="checkbox"/>	NONE	<input type="checkbox"/>	PI LETTER
	<input type="checkbox"/>	WARNING LETTER	<input type="checkbox"/>	REJECTION OF STUDY

DATE EIR REC'D DSI: 10/18/01
DATE EIR REC'D BY REVIEWER: 11/13/01
1ST DRAFT REVIEW COMPLETED: 01/18/02

This review evaluates the referenced Establishment Inspection Report (EIR) and determines the FDA's final classification. CDER's Division of Scientific Investigations (HFD-45), FDA review divisions, and FDA field personnel use this information both for determining action on the audited non-clinical toxicity studies and for monitoring this non-clinical testing facility.

The master schedule collected during the inspection was used by Investigator Austin to select the following studies for data audits:

STUDIES AUDITED DURING THIS INSPECTION:

1. NDA: 21-416
INDs: _____
SPONSOR: The final report says Bristol-Myers Squibb is the sponsor of the study, but the sponsor of the above referenced NDA _____ is Otsuka.
DRUG: BMS-37039 (abilitat, aripiprazole, OPC-31, OPC-14597)
REV DIV: HFD-120
STUDY #: 99353
STUDY TITLE: BMS-37039: Twenty-Six-Week Oral Toxicity Study in Rats
FINAL REPORT DATE: 03/20/01
2. IND: _____
SPONSOR: Bristol-Myers Squibb
DRUG: BMS-299897
REV DIV: HFD-120
STUDY #: DM00003
STUDY TITLE: BMS-299897: One-Month Oral Toxicity Study in Dogs
FINAL REPORT DATE: unknown
3. INDs: _____
SPONSOR: Bristol-Myers Squibb
DRUG: BMS-232632
REV DIV: HFD-530
STUDY #: 99323
STUDY TITLE: BMS-232632, Three Month Oral Range Finding Study in Mice
FINAL REPORT DATE: 01/04/00
4. INDs: _____
SPONSOR: Bristol-Myers Squibb
DRUG: BMS-309403
REV DIV: HFD-510
STUDY #: DM00024
STUDY TITLE: BMS-309403, One-Year Oral Toxicity Study in Dogs
FINAL REPORT DATE: This was an on-going study.

5. IND: _____
SPONSOR: Bristol-Myers Squibb
DRUG: BMS-356103
REV DIV: HFD-510
STUDY #: BM01004
STUDY TITLE: BMS-356103, Six-Month Oral Toxicity Study
in Rats
FINAL REPORT DATE: This was an on-going study.

BACKGROUND:

This pharmaceutical company was incorporated on August 11, 1933, in Delaware as Bristol-Myers Inc. It took its present corporate name in approximately October of 1990 after acquiring Squibb.

As a sponsor and testing facility, it conducts certain non-clinical laboratory (toxicity) studies that are shorter than 6 months. As a sponsor, it contracts longer studies out to other non-clinical testing facilities.

The last five GLP inspections at this facility known to CDER resulted in the following final classifications:

FY	DONE	TYPE	CLASS.	CENTER
3/91	06/05/91	S&C	NAI	CDER
4/92	10/13/92	D	VAI-1	CDER
3/93	06/30/93	S	NAI	CDER
4/96	07/22/96	S&D	NAI	CDER
4/98	01/13/99	S&D	NAI	CDER

DISCUSSION:

The EIR consists of a summary of findings, discussion of the audited studies, and reviews of the firm, its personnel, in house quality assurance unit, facilities, facility operations, equipment, reagents and solutions, animal care, master schedule, computer usage, analysis of test and control articles, care of test and control articles, and maintenance of records and reports. To the EIR are appended 14 exhibits, including the master schedule, protocols for the 5 audited studies, portions of final reports for the 3 completed studies that were audited, selected indexes of SOPs, and organizational charts for the firm.

Investigator Austin noted no basic departures from protocols or GLPs during the facility inspection and the review of the selected studies. No Form FDA-483 was issued.

The organizational charts that were collected do not include persons who support computerized operations. During the next inspection, such charts should be collected; and the records of education, training, and experience of these persons should be evaluated.

During this inspection, minutes of the last two meetings of the Institutional Animal Care and Use Committee (IACUC) were reviewed. During the next inspection, SOPs for the IACUC should be collected as exhibits, along with the most recent committee minutes.

RECOMMENDATIONS:

- 1> The completed studies that were audited are acceptable for review in support of regulatory decisions by the Agency.
- 2> Routinely reinspect in two years, as Agency resources permit.

RECOMMENDED HQ CLASSIFICATION: NAI

Reviewer: _____ /S/ _____
Charles A. Snipes, Ph.D.
Pharmacologist

Supervisory Concurrence:

Concur: CTV Date: 01/29/02

Nonconcurrency: _____ : Date: _____

(See attached supervisory memorandum)

FEI : 1831144
Assignment Number : 176026
Inspection Conclusion : NAI (No Action Indicated)
District Recommendation : NAI
Final HQ Classification : NAI

cc:

HFA-224
HFD-45
HFD-48/Fujiwara(3)/Snipes/CF
HFD-120/NDA 21436/Freed/Hardeman
HFD-120/reed/Hardeman
HFD-120/reed/Hardeman
HFD-120/reed/Hardeman
HFD-120/reed/Hardeman
HFD-510/ao/Cross
HFD-510/oirangle/Cross
HFD-510/ao/Cross
HFD-120/sher/Fanari
HFD-530/uu/Young
HFD-530/uu/Young
HFR-CE700
HFR-CE750/Bellamy
HFR-CE7545/Austin
DSI/GBIB: BMS-5-Rev

**APPEARS THIS WAY
ON ORIGINAL**