

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-782

PHARMACOLOGY REVIEW



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

MEMO TO FILE

NDA#: 21-782
Review Number: 2
Serial #: N 000 (21-Sep-2004), N 000 BP (16-Nov-2004), N 000 BZ (22-Feb-2005), N 000 BP (07-Mar-2005)
Drug: Ramelteon [ROZEREM™]
Indication: Treatment of insomnia
Sponsor: Takeda Global R&D
Stamp Date: See above
Division: Anesthesia, Analgesia and Rheumatology Products (HFD-170)
Date of Memo: July 22, 2005

Background

This memo to the file of NDA 21-782 (Ramelteon [ROZEREM™]) is for the purpose of addressing two changes to the original pharmacology/toxicology NDA review:

- 1) The result of studies of ramelteon in the chromosomal aberration assay will be considered to have demonstrated a lack of clastogenicity in the absence of S9 metabolic activation though the positive clastogenic finding in the presence of S9 remains.
- 2) The Product Information label has been revised after negotiations with the Sponsor.

Item 1: Chromosomal aberration results

The original chromosomal aberration assay is summarized in the following tables:

Appears This Way
On Original

Test for Induction of:	Chromosome aberration	No. of Independent Assays:	1	Test Article:	TAK-375
Strains:	Chinese hamster lung cells	No. of Replicate Cultures:	2	Study Report No.:	3644.M-11-17-1
Metabolizing System:	Phenobarbital and 5, 6-benzoflavone-induced rat liver S9 5*	No. of Cells Analyzed/Cultures:	100	GLP Compliance:	Yes
Vehicles:	For Test Article: DMSO	For Positive Controls:	Saline	Date of Treatment:	November, 1997
Treatment:	Continuous treatment for 24 and 48 hours without S9, pulse treatment for 6 hours with 18-hour recovery time with and without S9				
Cytotoxic Effects:	Dose-related decreases in cell survival				
Genotoxic Effects:	Chromosome aberrations at 2113 µg/mL (48 hour treatment) and at 450 µg/mL with S9, concurrent with decreased cell survival rate and/or decreased mitotic indices.				

Metabolic Activation	Test Article	Concentration (µg/mL)	Exposure-Expression Time (hr)	Cytotoxicity (% of control) (a)	Cytotoxicity (% of control) (b)	Aberrant Cells Mean%	Polyloid Cells % (c)	Judgement (d)
Without Activation	DMSO	0	24-0	100	100	0.0	1.0	-
	TAK-375	56.3	24-0	97.6	76.7	1.5	2.1	-
		113	24-0	90.7	63.3	3.5	1.2	-
		225	24-0	67.6	26.7	4.0	2.2	-
		450 (e)	24-0	7.9	0.0	ND	ND	NA
	Mitomycin C	0.05	24-0	ND	ND	43.0	ND	+
Without Activation	DMSO	0	48-0	100	100	1.0	0.9	-
	TAK-375	56.3	48-0	89.1	73.0	2.0	2.0	-
		113	48-0	65.8	73.0	5.5	3.3	±
		225	48-0	42.2	20.6	9.0	2.7(0)	±
		450 (e)	48-0	5.0	0.0	ND	ND	NA
	Mitomycin C	0.025	48-0	ND	ND	40.0	ND	-

Metabolic Activation	Test Article	Concentration (µg/mL)	Exposure-Expression Time (hr)	Cytotoxicity (% of control) (a)	Cytotoxicity (% of control) (b)	Aberrant Cells Mean%	Polyloid Cells % (c)	Judgement (d)
Without Activation	DMSO	0	6-18	100	100	0.5	1.0	-
	TAK-375	56.3	6-18	97.8	80.4	0.5	1.5	-
		113	6-18	100.2	94.1	1.0	2.1	-
		225	6-18	82.0	98.0	0.0	1.4	-
		450 (e)	6-18	9.5	7.8	ND	ND	NA
	Cyclophosphamide	12.5	6-18	ND	ND	0.0	ND	-
With Activation	DMSO	0	6-18	100	100	0.5	0.8	-
	TAK-375	113	6-18	93.6	107.2	0.0	0.9	-
		225	6-18	86.8	116.9	0.5	0.8	-
		450 (e)	6-18	50.9	63.9	11.5	3.5	-
		900 (e)	6-18	10.4	4.8	ND	ND	NA
	Cyclophosphamide	12.5	6-18	ND	ND	39.5	ND	-

NA indicates Not applicable, ND, Not determined.

(a) Based on cell survival.

(b) Based on mitotic indices.

(c) Based on 1000 metaphases.

(d) Judgement was made according to the total (%) of structural aberrations and polyloid cells. - negative (both values are <5%) = inconclusive (either of the values is ≥5% and <10%), +, positive (either of the values is ≥10%)

(e) Precipitation at the end of treatment.

(f) Based on 926 metaphases.

The results of ramelteon exposure in the S9 metabolic activation condition (6 hr exposure/18 hr expression) using Chinese hamster lung (CHL) cells were unambiguously positive for clastogenicity in this assay. Several experiments were conducted to assess ramelteon in this assay in the absence of S9 metabolic activation (6-18; 24-0; 48-0 as above). Both the 6-18 and 24-0 exposure-expression conditions yielded inconclusive results due to unacceptably low cytotoxicity (i.e. > 50% cell survival) except at the highest concentrations used in each condition at which severe cytotoxicity precluded analysis. Note that with cell lines, the use of mitotic index as a measure of cytotoxicity is inappropriate; thus, only the measure based on cell survival is useful (i.e. Cytotoxicity column [a]). The Sponsor conducted a further experiment with a 48 hr ramelteon exposure (48-0) and observed a clastogenic response which they defined as equivocal and which this reviewer considered to be unambiguously positive due to dose-response and a 9-fold elevation above vehicle control at concentrations which did not produce a precipitate. Therefore the version of the product information label for ramelteon included in revised form in the original NDA review indicated ramelteon demonstrated

clastogenicity in the chromosomal aberration assay both in the presence and absence of S9 metabolic activation.

Subsequent to this review, the Sponsor requested the Division consider a subsequent chromosomal aberration study (M-11-00354) using a ramelteon lot (Lot# M375-017) fortified with the impurity [] of the drug substance. This study is summarized in the following tables:

Test for Induction of:	Chromosome aberration	No. of Independent Assays:	1	Test Article:	TAK-375
Strain:	Chinese hamster lung cells	No. of Replicate Cultures:	2	Study/Report No.:	SR01049 M-11-00354
Metabolizing System:	Phenobarbital and 5, 6-benzoflavone-induced rat liver S9 S ₉	No. of Cells Analyzed/Cultures:	100	CLP Compliance:	Yes
Vehicles:	For Test Article:	DMSO	For Positive Controls:	Saline for mitomycin C; DMSO for benzo[a]pyrene	Date of Treatment: November, 2001
Treatment:	Continuous treatment for 24 hours; without S9; pulse treatment for 6 hours; with 18-hour recovery time with and without S9				
Cytotoxic Effects:	Decreases in growth rate				
Genotoxic Effects:	None				

Metabolic Activation	Test Article	Concentration (µg/mL)	Exposure-Expression Time (hr)	Cytotoxicity (% of control) (a)	Cytotoxicity (% of control) (b)	Aberrant Cells Mean%	Polyploid Cells % (c)	Judgement (d)
Without Activation	DMSO	0	24-0	100	100	2.0	0.4	-
	TAK-375	209.7	24-0	54.5	112.5	0.0	0.0	-
		262.1	24-0	53.5	65.0	1.0	0.0	-
		327.7	24-0	43.0	50.0	0.0	0.1	-
		409.6	24-0	ND	ND	50.5	ND	-
Mitomycin C	0.05	24-0	ND	ND	50.5	ND	-	
Without Activation	DMSO	0	6-18	100	100	2.5	0.4	-
	TAK-375	262.1	6-18	95.0	128.0	0.5	0.1	-
		327.7	6-18	88.5	154.0	1.0	0.0	-
		409.6	6-18	75.5	110.0	0.5	0.1	-
		512	6-18	17.0	NC	ND	ND	NA
	Mitomycin C	0.1	6-18	ND	ND	43.0	ND	-

Metabolic Activation	Test Article	Concentration (µg/mL)	Exposure-Expression Time (hr)	Cytotoxicity (% of control) (a)	Cytotoxicity (% of control) (b)	Aberrant Cells Mean%	Polyploid Cells % (c)	Judgement (d)
With Activation	DMSO	0	6-18	100	100	1.0	0.1	-
	TAK-375	327.7	6-18	98.5	79.6	2.0	0.0	-
		409.6	6-18	93.5	85.7	1.0	0.0	-
		512	6-18	99.5	73.5	1.5	0.1	-
		640	6-18	19.0	NC	ND	ND	NA
	Benzo[a]pyrene	10	6-18	ND	ND	36.5	ND	-

NA indicates Not applicable; NC, No observable cells; ND, Not determined; -, negative (both values are < 5%); ±, inconclusive (either of the values is ≥ 5% and < 10%); +, positive (either of the values is ≥ 10%).

(a) Based on growth rate.

(b) Based on mitotic indices.

(c) Based on 1000 metaphases.

(d) Judgment was made according to the total (%) of structural aberrations and polyploid cells.

The experiment in which exposure of CHL cells to ramelteon was for 24 hr (24-0) in the absence of S9 metabolic activation reached a dose level producing adequate cytotoxicity (≥ 50%) and did not detect an increase in chromosomal aberrations. This reviewer considered the study to be valid and evidence that ramelteon was not clastogenic in the absence of metabolic activation. This assessment along with supportive materials was sent to the Associate Director for Pharmacology Toxicology, Dr. David Jacobson-Kram, for evaluation. Dr. Jacobson-Kram's opinion is excerpted below from an e-mail communication:

I agree with your assessment. The study is positive in the presence of S9 and negative in the absence. Also keep in mind that the 48 hour continuous exposure is not part of the ICH guidance. It is not clear why the sponsor did it. Perhaps it's an old study with a new date.

With concurrence from Dr. Jacobson-Kram and further internal discussion within the Division the decision was made to remove the reference to a positive genotoxic result in

the absence of S9 metabolic activation from the product information label. The positive result in the presence of S9 metabolic activation, however, remains in the label.

Item 2: Final negotiated Product Information label (Pharm/Tox Sections only)

[Clinical Pharmacology]

Pharmacodynamics and Mechanism of Action

ROZEREM (ramelteon) is a melatonin receptor agonist with both high affinity for melatonin MT₁ and MT₂ receptors and selectivity over the MT₃ receptor. Ramelteon demonstrates full agonist activity *in vitro* in cells expressing human MT₁ or MT₂ receptors, and high selectivity for human MT₁ and MT₂ receptors compared to the MT₃ receptor.

The activity of ramelteon at the MT₁ and MT₂ receptors is believed to contribute to its sleep-promoting properties, as these receptors, acted upon by endogenous melatonin, are thought to be involved in the maintenance of the circadian rhythm underlying the normal sleep-wake cycle.

Ramelteon has no appreciable affinity for the GABA receptor complex or for receptors that bind neuropeptides, cytokines, serotonin, dopamine, noradrenaline, acetylcholine, and opiates. Ramelteon also does not interfere with the activity of a number of selected enzymes in a standard panel.

The major metabolite of ramelteon, M-II, is active and has approximately one tenth and one fifth the binding affinity of the parent molecule for the human MT₁ and MT₂ receptors, respectively, and is 17 – 25-fold less potent than ramelteon in *in vitro* functional assays. Although the potency of M-II at MT₁ and MT₂ receptors is lower than the parent drug, M-II circulates at higher concentrations than the parent producing 20 – 100 fold greater mean systemic exposure when compared to ramelteon. M-II has weak affinity for the serotonin 5-HT_{2B} receptor, but no appreciable affinity for other receptors or enzymes. Similar to ramelteon, M-II does not interfere with the activity of a number of endogenous enzymes

All other known metabolites of ramelteon are inactive.

[Precautions]

Carcinogenesis, Mutagenesis, and Impairment of Fertility

Carcinogenesis

In a two-year carcinogenicity study, B6C3F₁ mice were administered ramelteon at doses of 0, 30, 100, 300, or 1000 mg/kg/day by oral gavage. Male mice exhibited a dose-related increase in the incidence of hepatic tumors at dose levels ≥ 100 mg/kg/day including hepatic adenoma, hepatic carcinoma, and hepatoblastoma. Female mice

developed a dose-related increase in the incidence of hepatic adenomas at dose levels $\geq$ 300 mg/kg/day and hepatic carcinoma at the 1000 mg/kg/day dose level. The no-effect level for hepatic tumors in male mice was 30 mg/kg/day (103-times and 3-times the therapeutic exposure to ramelteon and the active metabolite M-II, respectively, at the maximum recommended human dose [MRHD] based on an area-under-the-curve [AUC] comparison). The no-effect level for hepatic tumors in female mice was 100 mg/kg/day (827-times and 12-times the therapeutic exposure to ramelteon and M-II, respectively, at the MRHD based on AUC).

In a two-year carcinogenicity study conducted in the Sprague-Dawley rat, male and female rats were administered ramelteon at doses of 0, 15, 60, 250 or 1000 mg/kg/day by oral gavage. Male rats exhibited a dose-related increase in the incidence of hepatic adenoma and benign Leydig cell tumors of the testis at dose levels $\geq$ 250 mg/kg/day and hepatic carcinoma at the 1000 mg/kg/day dose level. Female rats exhibited a dose-related increase in the incidence of hepatic adenoma at dose levels $\geq$ 60 mg/kg/day and hepatic carcinoma at the 1000 mg/kg/day dose level. The no-effect level for hepatic tumors and benign Leydig cell tumors in male rats was 60 mg/kg/day (1,429-times and 12-times the therapeutic exposure to ramelteon and M-II, respectively, at the MRHD based on AUC). The no-effect level for hepatic tumors in female rats was 15 mg/kg/day (472-times and 16-times the therapeutic exposure to ramelteon and M-II, respectively, at the MRHD based on AUC).

The development of hepatic tumors in rodents following chronic treatment with non-genotoxic compounds may be secondary to microsomal enzyme induction, a mechanism for tumor generation not thought to occur in humans. Leydig cell tumor development following treatment with non-genotoxic compounds in rodents has been linked to reductions in circulating testosterone levels with compensatory increases in luteinizing hormone release, which is a known proliferative stimulus to Leydig cells in the rat testis. Rat Leydig cells are more sensitive to the stimulatory effects of luteinizing hormone than human Leydig cells. In mechanistic studies conducted in the rat, daily ramelteon administration at 250 and 1000 mg/kg/day for 4 weeks was associated with a reduction in plasma testosterone levels. In the same study, luteinizing hormone levels were elevated over a 24 hour period after the last ramelteon treatment; however, the durability of this luteinizing hormone finding and its support for the proposed mechanistic explanation was not clearly established.

Although the rodent tumors observed following ramelteon treatment occurred at plasma levels of ramelteon and M-II in excess of mean clinical plasma concentrations at the MRHD, the relevance of both rodent hepatic tumors and benign rat Leydig cell tumors to humans is not known.

Reviewer's comment: NOAEL safety margins were used in the label rather than LOAEL exposure margins at the dose levels associated with tumor development. There is no stated CDER policy on the preferential use of NOAELs or LOAELs in this section of the label. This reviewer believes that NOAELs are more informative to the prescribing physician and therefore more appropriate. This

issue was raised with Dr. Abigail Jacobs, Associate Director for Pharmacology/Toxicology (ODE IV & IV) and a member of the Executive Carcinogenicity Assessment Committee, who believed the NOAEL safety margin should be used in the label though the exposure margins at the doses associated with tumors could also be given. Dr. David Jacobson-Kram, Associate Director of Pharmacology/Toxicology and the chair of the Executive Carcinogenicity Assessment Committee, observed that typically the LOAEL exposure margins are given in the label but stated that the NOAEL safety margins are "at least as relevant".

Mutagenesis

Ramelteon was not genotoxic in the following: *in vitro* bacterial reverse mutation (Ames) assay; *in vitro* mammalian cell gene mutation assay using the mouse lymphoma TK⁺ cell line; *in vivo/in vitro* unscheduled DNA synthesis assay in rat hepatocytes; and in *in vivo* micronucleus assays conducted in mouse and rat. Ramelteon was positive in the chromosomal aberration assay in Chinese hamster lung cells in the presence of S9 metabolic activation.

Separate studies indicated that the concentration of the M-II metabolite formed by the rat liver S9 fraction used in the *in vitro* genetic toxicology studies described above exceeded the concentration of ramelteon; therefore, the genotoxic potential of the M-II metabolite was also assessed in these studies.

Impairment of Fertility

Ramelteon was administered to male and female Sprague-Dawley rats in an initial fertility and early embryonic development study at dose levels of 6, 60, or 600 mg/kg/day. No effects on male or female mating or fertility were observed with a ramelteon dose up to 600 mg/kg/day (786-times higher than the MRHD on a mg/m² basis). Irregular estrus cycles, reduction in the number of implants, and reduction in the number of live embryos were noted with dosing females at ≥ 60 mg/kg/day (79-times higher than the MRHD on a mg/m² basis). A reduction in the number of corpora lutea occurred at the 600 mg/kg/day dose level. Administration of ramelteon up to 600 mg/kg/day to male rats for 7 weeks had no effect on sperm quality and when the treated male rats were mated with untreated female rats there was no effect on implants or embryos. In a repeat of this study using oral administration of ramelteon at 20, 60 or 200 mg/kg/day for the same study duration, females demonstrated irregular estrus cycles with doses ≥ 60 mg/kg/day, but no effects were seen on implantation or embryo viability. The no-effect dose for fertility endpoints was 20 mg/kg/day in females (26-times the MRHD on a mg/m² basis) and 600 mg/kg/day in males (786-times higher than the MRHD on a mg/m² basis) when considering all studies.

Pregnancy: Pregnancy Category C

Ramelteon has been shown to be a developmental teratogen in the rat when given in doses 197 times higher than the maximum recommended human dose [MRHD] on a mg/m² basis. There are no adequate and well-controlled studies in pregnant women.

Ramelteon should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

The effects of ramelteon on embryo-fetal development were assessed in both the rat and rabbit. Pregnant rats were administered ramelteon by oral gavage at doses of 0, 10, 40, 150, or 600 mg/kg/day during gestation days 6-17, which is the period of organogenesis in this species. Evidence of maternal toxicity and fetal teratogenicity was observed at doses greater than or equal to 150 mg/kg/day. Maternal toxicity was chiefly characterized by decreased body weight and, at 600 mg/kg/day, ataxia and decreased spontaneous movement. At maternally toxic doses (150 mg/kg/day or greater), the fetuses demonstrated visceral malformations consisting of diaphragmatic hernia and minor anatomical variations of the skeleton (irregularly shaped scapula). At 600 mg/kg/day, reductions in fetal body weights and malformations including cysts on the external genitalia were additionally observed. The no-effect level for teratogenicity in this study was 40 mg/kg/day (1,892-times and 45-times higher than the therapeutic exposure to ramelteon and the active metabolite M-II, respectively, at the MRHD based on an area-under-the-curve [AUC] comparison). Pregnant rabbits were administered ramelteon by oral gavage at doses of 0, 12, 60, or 300 mg/kg/day during gestation days 6-18, which is the period of organogenesis in this species. Although maternal toxicity was apparent with a ramelteon dose of 300 mg/kg/day, no evidence of fetal effects or teratogenicity was associated with any dose level. The no-effect level for teratogenicity was, therefore, 300 mg/kg/day (11,862-times and 99-times higher than the therapeutic exposure to ramelteon and M-II, respectively, at the MRHD based on AUC). The effects of ramelteon on pre- and post-natal development in the rat were studied by administration of ramelteon to the pregnant rat by oral gavage at doses of 0, 30, 100, or 300 mg/kg/day from day 6 of gestation through parturition to postnatal (lactation) day 21, at which time offspring were weaned. Maternal toxicity was noted at doses of 100 mg/kg/day or greater and consisted of reduced body weight gain and increased adrenal gland weight. Reduced body weight during the post-weaning period was also noticed in the offspring of the groups given 100 mg/kg/day and higher. Offspring in the 300 mg/kg/day group demonstrated physical and developmental delays including delayed eruption of the lower incisors, a delayed acquisition of the righting reflex, and an alteration of emotional response. These delays are often observed in the presence of reduced offspring body weight but may still be indicative of developmental delay. An apparent decrease in the viability of offspring in the 300 mg/kg/day group was likely due to altered maternal behavior and function observed at this dose level. Offspring of the 300 mg/kg/day group also showed evidence of diaphragmatic hernia, a finding observed in the embryo-fetal development study previously described. There were no effects on the reproductive capacity of offspring and the resulting progeny were not different from those of vehicle-treated offspring. The no-effect level for pre- and postnatal development in this study was 30 mg/kg/day (39-times higher than the MRHD on a mg/m² basis).

Labor and Delivery

The potential effects of ROZEREM on the duration of labor and/or delivery, for either the mother or the fetus, have not been studied. ROZEREM has no established use in labor and delivery.

Nursing Mothers

Ramelteon is secreted into the milk of lactating rats. It is not known whether this drug is excreted in human milk. No clinical studies in nursing mothers have been performed. The use of ROZEREM in nursing mothers is not recommended.

Supervisor's Comment (R. Daniel Mellon, Ph.D.): Although the above wording is not consistent with that provided in the Code of Federal Regulations 21CFR §201.57, the review team agreed with the Sponsor's proposal that due to the potential effects of ramelteon on testosterone and prolactin, the use of this drug in nursing mothers should be described as not recommended.

Adam M. Wasserman, Ph.D.

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/s/

Adam Wasserman
7/22/05 01:28:25 PM
PHARMACOLOGIST

R. Daniel Mellon
7/22/05 01:41:22 PM
PHARMACOLOGIST
I concur.

MEMORANDUM

July 13, 2005

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-782

I concur that this application may be approved based on the review of the pharmacology/toxicology data prepared by Dr. Adam Wasserman. Specifically, I agree that Pregnancy Category C is appropriate based on exposure multiples as given in the product label. Although the high incidence of hepatic tumors observed in rodents is notable, these occurred at significant multiples of human exposure to the drug at therapeutic doses. I concur with Dr. Wasserman and Dr. R. Daniel Mellon that ramelteon is clastogenic and should be labeled as such.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluations II & III

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/s/

Kenneth Hastings
7/13/05 03:43:44 PM
PHARMACOLOGIST



FDA Center for Drug Evaluation and Research
Division of Anesthesia, Analgesia, and Rheumatology Products
HFD-170, Room 9B-45, 5600 Fishers Lane, Rockville, MD 20857
MEMORANDUM

Date: June 28, 2005
To: NDA File 21-782 (TAK-375/Ramelteon)
From: R. Daniel Mellon, Ph.D.
Supervisory Pharmacologist, DAARP
Subject: Secondary Pharmacology Toxicology Review
Date of Submission: September 22, 2004

Background: NDA 21-782 was submitted by Takeda Global R&D to support the marketing application of the drug ramelteon (TAK-375) for the indication of insomnia. Ramelteon has high affinity and selectivity for MT₁ and MT₂ melatonin receptors, which are thought to play a role in the regulation of sleep-wake cycles in humans and other mammals.

Takeda submitted IND 58,136 to the Division of Neuropharmacology Drug Products in 1999, and the primary pharmacology/toxicology reviewer for that Division was Dr. Aisar Atrakchi. When the IND was transferred to HFD-170 in the Fall of 2003, Dr. Adam Wasserman was assigned the IND and fulfilled the role of the primary pharmacology/toxicology reviewer. The NDA review has been completed by Dr. Wasserman. Dr. Wasserman's NDA review concludes that from the pharmacology and toxicology perspective, the NDA can be approved.

Recommendation: I concur with Dr. Wasserman's recommendation that the NDA can be approved from the pharmacology and toxicology perspective.

Summary and Evaluation of Key Issues:

Dr. Wasserman's review made the following recommendations for additional nonclinical studies:

Although several nonclinical studies should be performed by the Sponsor to address gaps in the knowledge of TAK-375, these are not considered to be approvability issues

and may be conducted as part of a Phase 4 commitment if approved on the first cycle. Otherwise these studies should be completed for a 2nd submission.

- 1. Full characterization of M-II in cardiovascular safety studies should be completed as in vitro studies with the exception of the hERG channel assay did not include this active metabolite and the in vivo studies submitted either would not be expected to evaluate M-II (monkey) or did not assess the level of this metabolite (dog).*
- 2. Characterization of the inactive metabolite M-IV should be completed with evaluation in safety pharmacology studies as well as completion of an intravenous toxicity study with the isolated metabolite in monkey, analogous to that done with M-II, in order to satisfy requirements for a non-rodent evaluation of toxicity.*
- 3. Repeat of the in vitro chromosomal aberration assay in CHL or other system to resolve methodological problems and confirm or refute the positive clastogenic response observed in the original study.*

I agree with Dr. Wasserman that the above studies, although not required for NDA approval, would be beneficial to provide a more complete characterization of the drug substance. However, I do not believe that a formal commitment from the Sponsor to complete the studies is required. My rationale is provided below:

- 1. Characterization of Metabolite M-II in Cardiovascular Safety Studies.** As noted by Dr. Wasserman, the submitted nonclinical cardiovascular safety pharmacology studies with TAK-375 do not provide a complete evaluation of the M-II metabolite. Although Dr. Wasserman's conclusion is correct, the Sponsor completed a thorough clinical QT Study testing multiple 32 mg and 64 mg doses of ramelteon (TL040). Provided the clinical study is adequate, no further non-clinical cardiovascular safety studies of the M-II metabolite would be required (see Dr. Elizabeth McNeil's Clinical review and Dr. David Lee's Clinical Pharmacology review for details of the study results).
- 2. Qualification of Metabolite M-IV.** Dr. Wasserman's review accurately notes that the human produces greater levels of the M-IV metabolite than the non-clinical species used to characterize the toxicity of TAK-375. As outlined in the recently released Draft Guidance for Industry: Safety Testing of Drug Metabolites (June 2005), the M-IV metabolite is considered a major human metabolite and should be adequately qualified. The requirement for safety qualification of this metabolite was not discussed with the Sponsor prior to NDA submission. Dr. Wasserman, therefore, indicated that evaluation of this metabolite could be completed post-approval. However, Dr. Wasserman determined that the rat toxicology studies provide a mean plasma concentration of M-IV at the NOAEL dose that establishes a margin of safety to support the NDA. In contrast, plasma concentrations of M-IV that produced no adverse effects in the monkey toxicology studies were below the mean plasma levels expected in the human at the maximum recommended daily dose. Plasma

concentrations of M-IV at the monkey LOAEL provided acceptable coverage to support the NDA application. Therefore, although not an ideal characterization, the existing toxicology studies do provide acceptable support for the safety of the M-IV metabolite. I agree with Dr. Wasserman that the Sponsor should pursue additional characterization of this metabolite post approval; however, I do not feel that the studies require a formal commitment from the Sponsor.

3. **Positive Genotoxicity Data.** The Sponsor concludes that the genetic toxicology data indicates that TAK-375 is nongenotoxic. The Sponsor's proposed description of the positive genetic toxicology finding in the package insert downplays the finding as likely due to cytotoxicity. However, as the Sponsor did not provide a mechanistic explanation for the positive result which would eliminate concern, the positive finding must be considered valid and therefore can not be dismissed. As such, the Sponsor's proposed description of the genotoxicity data in the package insert has been modified to include a description of all genetic toxicology study results. I agree with Dr. Wasserman's conclusion that the weight of evidence analysis suggests an overall lack of genotoxic hazard. Therefore, further studies are not required and the existing data can be described in the labeling. That being said, I agree that conducting additional genetic toxicology studies to determine the mechanism of the positive finding **may** support labeling that does not depict TAK-375 as a genotoxic carcinogen. Further characterization of the findings **may** favorably alter the risk-benefit analysis for the Agency and therefore may also provide benefit to the Sponsor. However, such studies are not required for NDA approval.

In addition to the recommendations discussed above, Dr. Wasserman's review identified several key issues that could impact the risk-benefit analysis of this drug product. The four most significant of these issues are summarized below:

1. **Characterization of the Human Specific Metabolite M-II.** Dr. Wasserman's review noted that the active metabolite M-II is the major circulating species in human, reaching plasma levels that are on average 25-fold higher than the parent compound. Although M-II is detected in the mouse, rat, and rabbit, the levels are far lower with respect to the parent compound in these species compared to the human. M-II is present in even lower levels in the monkey. Adequate preclinical safety qualification of TAK-375, therefore, must also evaluate the exposure margins for the M-II metabolite in the various species employed in the toxicology studies. Due to the lower levels of M-II found in the preclinical models, the exposure margins for M-II are significantly lower than the exposure margins for the parent compound. Dr. Wasserman has included exposure margins for both the parent compound and the M-II metabolite in the package insert. I agree that these data should be included in order to adequately present the findings that impact the overall risk-benefit analysis for the drug product.

2. **Hepatic Tumors in Mice.** TAK-375 treatment of B6C3F1 mice resulted in the dose-dependent increase in the incidence of hepatic adenomas, carcinomas, and hepatoblastomas in male mice and adenomas and carcinomas in female mice that greatly exceeded the incidence in historical control data. An apparent treatment-related increase in hepatic tumor findings in rodent carcinogenicity studies, as is the case with TAK-375, is not unusual in the setting of hepatic enzyme induction. In the absence of positive genetic toxicology data, drug-induced hepatic tumors in rodents are thought to be a secondary response to sustained adaptive responses of the liver and species specific. In the carcinogenicity studies for TAK-375, however, the magnitude of the increase was considered to be unusually high by the Executive Carcinogenicity Assessment Committee (eCAC). As a result, the eCAC concluded that the relevance of these tumors to humans can not be excluded and should be described in the labeling. The table below clearly illustrates the greater incidence of liver tumors compared to historical control values. The table was extracted from Dr. Wasserman's review:

**Appears This Way
On Original**

**Apparent TAK-375-Related Neoplasms in 104-Week Mouse Bioassay
(Combined Early and Terminal Sacrifice)**

	TAK-375 Dose (mg/kg/day; oral gavage)											
	Males						Females					
	0	30	100	300	1000†	Trend#	0	30	100	300	1000†	Trend#
n =	55	55	55	55	55		55	55	55	55	55	
LIVER												
<i>Adenoma</i>												
Incidence	20	24	32	50*	54*	Yes	10	14	14	52*	55*	Yes
%	36	44	58	91	98		18	25	25	95	100	
Historical Mean (max)‡	34.9% (max: 36.7%)						16.9% (max: 22%)					
Historical Mean (max)&	43.3 ± 9.9% (max: 53%)						35.0 ± 8.7% (max: 40%)					
<i>Carcinoma</i>												
Incidence	9	6	18	26*	50*	Yes	4	3	3	11	51*	Yes
%	16	11	33	47	91		7	5	5	20	93	
Historical Mean (max)‡	17.1% (max: 20.0%)						2.7% (max: 7.3%)					
Historical Mean (max)&	31.6 ± 10.1% (max: 42%)						23 ± 6.2% (max: 28%)					
<i>Hepatoblastoma</i>												
Incidence	0	0	1	3*	10*	Yes	0	0	0	1	3	No
%	0	0	2	5	18		0	0	0	2	5	
Historical Mean (max)‡												
Historical Mean (max)&	1.5 ± 1% (max: 2%)						0.5 ± 1% (max: 2%)					
<i>Combined liver tumors (adenoma and carcinoma)</i>												
Incidence	25	28	40*	54*	54*	Yes	13	16	17	53*	55*	Yes
%	45	51	73	98	98		24	29	31	96	100	
<i>Combined liver tumors (adenoma, carcinoma and hepatoblastoma) – same incidence as above</i>												
Incidence	25	28	40*	54*	54*	Yes	13	16	17	53*	55*	Yes
%	45	51	73	98	98		24	29	31	96	100	
†, mice at 1000 mg/kg/day dose level were sacrificed during Week 95 (all other groups Week 104) ‡, from Takeda [] ; B6C3F1 mice. Total number of tissues examined: 175 (♂), 225 (♀) &, from NTP Historical Database (1999) B6C3F1 mice, oral methylcellulose; 4 studies, total number of animals examined = 200 #, Significantly different from control group, Peto's positive trend test p < 0.005 [common tumors] or p < 0.025 [rare tumors] *, p < Significantly different from control group, Fisher's exact test; p < 0.01 [common tumors] or p < 0.025 [rare tumors]												

3. **Hepatic and Leydig Cell Tumors in Rats.** TAK-375 treatment of Sprague-Dawley rats resulted in a dose-dependent development of hepatic tumors including adenoma and carcinoma in both males and females. In addition, males treated with the high dose of TAK-375 had an increased incidence of Leydig cell tumors compared to control treated males. Although the Sponsor conducted studies that provide some support for a species specific mechanism of Leydig cell tumor formation, the evidence provided was not conclusive. As noted in the mouse studies, the magnitude of the TAK-375 associated increase in tumor incidence was considered to be unusually high by the Executive Carcinogenicity Assessment Committee (eCAC). As a result, the eCAC concluded that the relevance of these tumors to humans can not be excluded and should be described in the labeling. The table below clearly illustrates the greater incidence of liver and Leydig cell tumors in treated animals compared to historical control values. The table was extracted from Dr. Wasserman's review:

Apparent TAK-375-Related Neoplasms in 104-Week Rat Bioassay (Combined Early and Terminal Sacrifice)

Group	Males						Females					
	C1	C2	LD	Low MD	High MD	HD	C1	C2	LD	Low MD	High MD	HD [†]
Dose (mg/kg/day)	0	0	15	60	250	1000	0	0	15	60	250	1000
N	60	60	60	60	60	60	60	60	60	60	60	60
Liver												
<i>Adenoma, hepatocellular</i>												
Incidence	0 [§]	0	7	7	15 [‡]	47 [‡]	1 [§]	1	0	8 [‡]	22 [‡]	49 [‡]
%	0%	0%	12%	12%	25%	78%	2%	2%	0%	13%	37%	82%
Historical Control (Mean and Max) [#]	3.8% (max: 8.3%)						1.3% (max: 1.7%)					
Historical Control (Mean and Max) [@]	2.0% (max 8.0%)						1.6% (max 13.3%)					
<i>Carcinoma, hepatocellular</i>												
Incidence	0 [§]	0	0	0	0	38 [‡]	0 [§]	0	0	1	1	20
%	0%	0%	0%	0%	0%	63%	0%	0%	0%	2%	2%	33%
Historical Control (Mean and Max) [#]	0.8% (max: 1.7%)						0% (max: 0%)					
Historical Control (Mean and Max) [@]	2.2% (max 6.7%)						0.3% (max 1.7%)					

Testis						
Tumor, Leydig Cell						
Incidence	3 [§]	0	1	0	7	46 [‡]
%	5%	0%	2%	0%	12%	77 [¶] %
Historical Control (Mean and Max) [#]	1.7% (max: 2.5%)					N/A
Historical Control (Mean and Max) [@]	2.4% (max 9.3%)					
[†] , females at 1000 mg/kg/day were sacrificed during the 94 th week of administration [#] , From Takeda, $\left[\begin{array}{l} \text{ } \\ \text{ } \end{array} \right]$ database of spontaneous neoplasms in control [—] CD(SD)IGS rats in 104-week bioassays (3 studies, 240 animals/sex) [@] , From $\left[\begin{array}{l} \text{ } \\ \text{ } \end{array} \right]$ database of spontaneous neoplasms in control [—] CD@ (SD) rats in 104-week bioassays (31 studies; 12 [—] colony-derived); March 2004 publication. [§] , trend is significant (Peto's positive trend test) [‡] , significantly different from combined controls (p<0.01)						

As illustrated by Dr. Wasserman's table above, the incidence of both hepatic tumors and Leydig cell tumors clearly exceeds the historical control data. I concur with Dr. Wasserman and the conclusion of the eCAC, i.e., the relevance of these tumors to humans cannot be excluded and therefore should be reflected in the package insert.

- 4. Teratogenicity in Rats.** TAK-375 administration to pregnant rats during organogenesis resulted in teratogenic effects. TAK-375 treatment was associated with a dose-dependent increase in fetal malformations (diaphragmatic hernia and cysts on the external genitalia) as well as fetal variations (irregularly shaped scapula and ribs). Based on these findings, TAK-375 is a Pregnancy Category C drug. However, the plasma levels of TAK-375 that were associated with teratogenic effects in rats were far greater than the mean plasma levels obtained in humans treated with the maximum recommended human dose (MRHD) of TAK-375. As such, there is a 1,892-fold margin of safety margin based on a comparison of the AUC exposure to TAK-375 and a 45-fold margin of safety based on a pharmacokinetic comparison of the AUC exposure to the M-II metabolite. The Segment 2 study in rabbits did not detect evidence of teratogenicity following doses of TAK-375 that resulted in plasma levels 11,862-fold the mean plasma level of TAK-375 and 99-fold the mean plasma level of the M-II metabolite in humans treated with the maximum recommended human dose. Both Dr. Wasserman and I agree that, although TAK-375 was teratogenic in the rat under the conditions of the assay, there is a large exposure margin for TAK-375 based upon pharmacokinetic data. As the exposure margin for the M-II metabolite is significantly less than that for TAK-375, the label should include both margins to adequately represent the data.

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/s/

R. Daniel Mellon
6/28/05 10:10:39 PM
PHARMACOLOGIST



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-732
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 9/22/04
PRODUCT: TAK-375/Ramelteon
INTENDED CLINICAL POPULATION: Insomnia
SPONSOR: Takeda Global R&D
DOCUMENTS REVIEWED: Pharmacology Toxicology Submissions EDR
REVIEW DIVISION: Division of Anesthesia, Analgesia, and
Rheumatology Products (HFD-170)
PHARM/TOX REVIEWER: Adam M. Wasserman, Ph.D.
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob Rappaport, M.D.
PROJECT MANAGER: Sara Stradley

Date of review submission to Division File System (DFS): June 22, 2005

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From the pharmacology/toxicology perspective this NDA may be approved.

B. Recommendation for nonclinical studies

Although several nonclinical studies should be performed by the Sponsor to address gaps in the knowledge of TAK-375, these are not considered to be approvability issues and may be conducted as part of a Phase IV commitment if approved on the first cycle. Otherwise these studies should be completed for a 2nd submission.

1. Full characterization of M-II in cardiovascular safety studies should be completed as *in vitro* studies with the exception of the hERG channel assay did not include this active metabolite and the *in vivo* studies submitted either would not be expected to evaluate M-II (monkey) or did not assess the level of this metabolite (dog).
2. Characterization of the inactive metabolite M-IV should be completed with evaluation in safety pharmacology studies as well as completion of an intravenous toxicity study with the isolated metabolite in monkey, analogous to that done with M-II, in order to satisfy requirements for a non-rodent evaluation of toxicity.
3. Repeat of the *in vitro* chromosomal aberration assay in CHL or other system to resolve methodological problems and confirm or refute the positive clastogenic response observed in the original study.

C. Recommendations on labeling

Note: The following label is a preliminary revision of the Sponsor's proposed label. The final label will be negotiated with the Sponsor.

DESCRIPTION

[

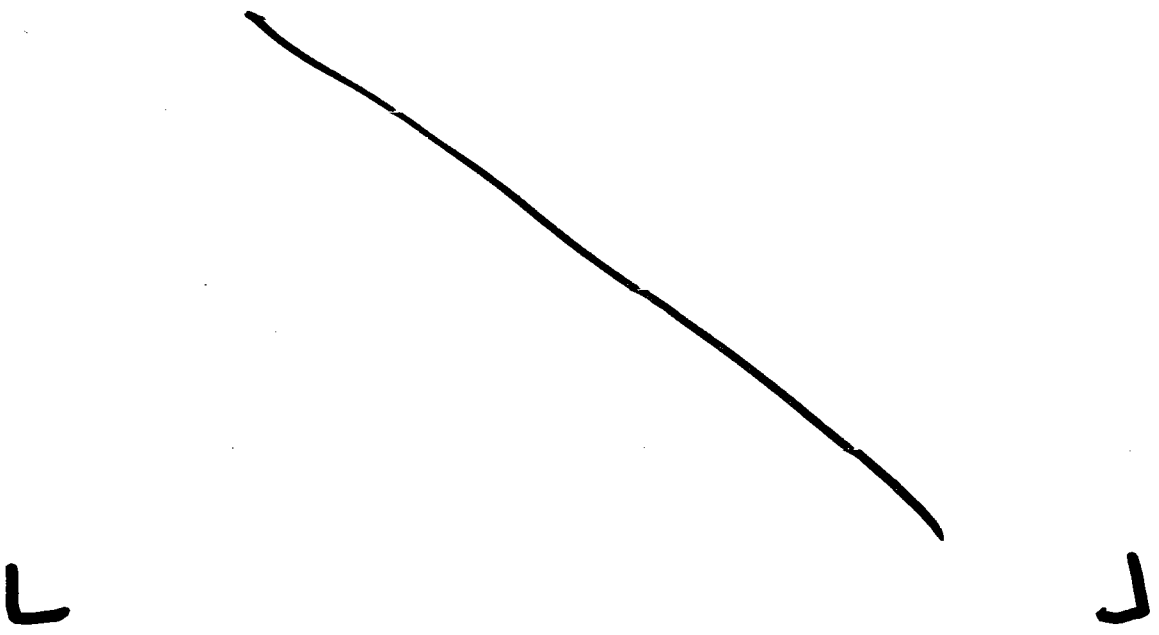
]

3 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

✓ _____ § 552(b)(5) Draft Labeling



II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Pharmacology:

TAK-375 and its active metabolite M-II were demonstrated by the Sponsor to be specific and high affinity agonists of the melatonin-1 (ML₁) class of melatonin receptors which comprise the mt₁ and mt₂ subtypes. Little affinity was exhibited at the melatonin receptor subtype designated MT3 which is a quinone reductase 2 enzyme. The K_d for TAK-375 at the mt₁, mt₂, and MT3 receptor was determined to be approximately 13 pM, 112 pM, and 2.65 μM, respectively. Therefore, TAK-375 has over 200,000-fold greater affinity for mt₁ and 23,000-fold greater affinity for mt₂ than MT3 and in addition demonstrates 6 – 16-fold greater affinity and potency for ML₁ receptors than melatonin itself. The M-II metabolite of TAK-375 has a similar high affinity for the ML₁ class of melatonin receptors though its affinity and potency is approximately 10 – 20-fold lower than the parent. TAK-375 and the active metabolite M-II have little affinity for other receptors and enzymes as determined in binding and functional screens of a large panel of potential alternate targets.

Pharmacodynamics:

Pharmacodynamic characterization of TAK-375 was conducted by administration of drug by the oral route to instrumented, freely-moving cats and rhesus macaque monkeys to characterize the potential hypnotic effects of this compound and more specifically on sleep parameters and architecture. Studies revealed oral administration of TAK-375 to produce decreased wakefulness, increased slow wave sleep and an increase in rapid eye movement (REM) sleep at a dose of 0.1 mg/kg in cats while administration of TAK-375 at doses ≥ 0.03

mg/kg in rhesus monkeys produced a decreased latency to light sleep and a decreased latency to slow wave sleep as well as a total increase in sleep duration. Sleep architecture was not observed to be altered nor was myorelaxation noted in contrast to zolpidem used as an active control. TAK-375 was also demonstrated to promote re-entrainment of circadian wheel running in rats subjected to an 8-hr phase advancement in their light/dark cycle when administered at doses ≥ 0.1 mg/kg p.o. This effect was similar to that found with melatonin at 10 mg/kg p.o. Other studies of TAK-375 pharmacodynamics demonstrated TAK-375 had no effect on behavioral assays of learning and memory, did not produce drug dependence or appear to provide reward, and has limited to no ability to produce self-administration behavior in monkeys. TAK-375 did not interact with diazepam on rota-rod performance in mice, a measure of motor coordination.

Safety Pharmacology:

Examination of TAK-375 in safety pharmacology studies revealed TAK-375 administered at high doses was capable of producing a profound CNS depression which could be lethal and was preceded by prone postures and respiratory abnormalities. TAK-375 did not reveal an ability to acutely disrupt cardiovascular, pulmonary or gastrointestinal function. No alterations in ECG waveforms were noted when tested in the conscious dog, no block of the hERG channel potassium current, and no prolongation of the action potential duration in sheep purkinje fibers were noted.

Pharmacokinetics:

TAK-375 is rapidly and passively absorbed from the GI tract but shows low oral bioavailability in all species tested ranging from 0.3% in the dog to 6.3% in the rat (human oral bioavailability is approximately 1.8%) due to extensive first pass hepatic metabolism. TAK-375 half-life is short in nonclinical species, <1 hr. TAK-375 has low plasma protein-binding and radiolabel studies demonstrate the radiolabeled drug or metabolites are distributed rapidly throughout the body with highest concentrations outside of the GI tract being in the liver, kidney, adrenal gland, and pituitary gland. Maximal concentrations in most tissues were achieved within 30 minutes following oral administration with levels declining rapidly 2 hr after oral administration and radioactivity was nearly absent in most tissues by 48 hr though radioactivity was retained longer in epididymal fat (tissue $t^{1/2}_{\beta}$ estimated at 60 hr versus <24 hr in all other tissues). In general, no evidence suggesting significant tissue accumulation or retention was demonstrated. Both TAK-375 and M-II rapidly penetrate the blood-brain-barrier when assessed in rats and enter the brain with levels that approximate the prevailing plasma concentration. TAK-375 and TAK-375 metabolites were found to cross the placenta and enter breast milk of rats as well.

TAK-375 is extensively metabolized by the liver as determined in *in vivo* studies in rats and monkeys, *in situ* studies in rats and *in vitro* studies with hepatic microsomes. Metabolites identified in the plasma and excreta of the rat were M-I, M-II, M-III, M-IV, M-V, M-VI, M-VII, and M-VIII. M-IX was detected in the bile of the rat. Studies in monkey demonstrated a similar metabolite profile, except M-V and M-IX were not found and M-VI and M-VIII were only detected in the urine. The only metabolite formed in human that was not observed in any nonclinical species was M-VIII-glucuronide. The major pathways of metabolism involved the hydroxylation of the propionamide side chain, forming M-II, and the oxidation of the ring hydroxyl group to generate a carbonyl group and form M-IV.

Another major metabolic pathway involved cleavage of the dihydrofuran ring of TAK-375 to form M-I. Other pathways typically involved ring or side chain oxidation. All metabolites other than M-II are believed inactive. *In vitro* study of the metabolism of TAK-375 by hepatic microsomal preparations from rat, dog, monkey and human liver indicate the predominant CYP involved is CYP1A2 which metabolizes TAK-375 to M-II with lesser involvement of CYP2C9 and CYP3A4 in the generation of subsequent metabolites. TAK-375 does not appear to be an inhibitor of CYP P450 enzymes; however, repeated exposure to rodents by the oral route is associated with significant hepatic enzyme induction with a pattern and magnitude similar to phenobarbital.

TAK-375-associated radiolabel is excreted principally in the urine with fecal routes playing a lesser role and limited respiratory excretion. Studies in a bile duct-cannulated rat suggest TAK-375-related compounds present in the feces are almost entirely due to biliary excretion. Very little unchanged TAK-375 is excreted and therefore the major method of TAK-375 elimination is through hepatic metabolism.

Repeat-dose toxicology:

The nonclinical toxicology program consisted of repeat-dose toxicity studies of up to 13 weeks duration in mice, 26 weeks in rats and 39-weeks in monkey. Dogs were not utilized due to the near complete first pass effect and other differences in metabolism when compared to humans. The Sponsor additionally evaluated TAK-375 in genetic toxicology assays, 2-year carcinogenicity bioassays in mice and rats, and a full reproductive toxicology assessment using rat and rabbit as models.

Chronic treatment with TAK-375 produced evidence of CNS toxicity in all species when given at a relatively high dose. In the mouse, decreased spontaneous locomotor activity was noted at a dose level ≥ 300 mg/kg/day while oligopnea was observed in males at and above this dose though in females it was seen only at 1000 mg/kg/day. Mice also exhibited prone or lateral position at a dose level ≥ 300 mg/kg/day in males and at 1000 mg/kg/day in females. These responses fully resolved within several days of treatment. In the rat, decreased spontaneous locomotor activity was also noted at doses ≥ 600 mg/kg/day in both Sprague-Dawley and Wistar strains. Salivation was observed with doses ≥ 40 mg/kg/day but this was considered to be due to the bitterness of the test article and therefore not a manifestation of toxicity. Administration of TAK-375 to the monkey produced a reduction in spontaneous locomotion when measured 1 hr post administration of dose levels of ≥ 12 mg/kg/day and which were observed throughout the 39-week study. An increased incidence and severity of emesis was also commonly observed in monkey treated with doses ≥ 50 mg/kg/day. Convulsions were seen in 2 male monkeys at the 200 mg/kg/day dose level on a single occasion and were preceded by observations of decreased muscle tone and oligopnea. These animals did not exhibit convulsive behavior subsequently.

Hematologic changes that were notable or common between species included an increase in platelets observed across species. In mice this occurred with dosing ≥ 1000 mg/kg/day while in rat an increase was noted with TAK-375 doses > 150 mg/kg/day in females in the chronic study and at ≥ 600 mg/kg/day in both sexes in a shorter 13-week study. Although no statistically significant changes in platelets were found when analyzed by group in monkeys, 2/4 high dose (200 mg/kg/day) males had platelet counts that were above the historical data range. Female cynomolgus monkeys did not show this effect at the highest dose studied. Mild reductions ($<5\%$) in red blood cell parameters (RBC, hemoglobin

and hematocrit) were noted in rats in the chronic study at the maximum dose level of 150 mg/kg/day and were indicative of a mild anemia with consequent histopathologic finding of splenic cell proliferation and increased spleen weight. This observation was not noted in either mouse or monkey animal models. In the chronic 39-week study of TAK-375 in the monkey, a dose-related but mild decrease in APTT was statistically significant at the 200 mg/kg/day level. Although dismissed by the Sponsor, 4/8 animals at this dose level were reported to have values below the historical control range.

Increased serum transaminases AST and ALT were noted in the mouse with dosing $\geq$ 1000 mg/kg/day though these increases were generally less than 2-fold. Total cholesterol was found to be significantly lower than control levels following treatment with TAK-375 300 – 1000 mg/kg/day in the 13-week study, however, total cholesterol was significantly increased at 1500 mg/kg/day in the 4 week toxicity study. A general reduction in the A/G ratio was noted in all studies at 1000 mg/kg/day and was perhaps related to decreased plasma albumin levels in males at 1000 mg/kg/day and females at $\geq$ 300 mg/kg/day.

In the rat, total cholesterol was increased with doses $\geq$ 150 mg/kg/day in females and 600 mg/kg/day in males and the elevation was less than 2-fold. A mildly decreased A/G ratio was noted in females only at the highest dose used in the chronic 26-week study (150 mg/kg/day). Total protein was increased, however, in both males and females at a dose $\geq$ 150 mg/kg/day with males having an increase in albumin levels. Plasma calcium was also noted to be increased in both males and females relative to control with a dose $\geq$ 150 mg/kg/day.

Repeat-dose toxicity studies of 4 weeks or 39-weeks duration in the monkey revealed several treatment-related changes in clinical chemistry. Elevated ALT was observed with dosing at 200 mg/kg/day in both studies though in the chronic 39-week study this was more marked in males and became more severe over the course of the study. ALT was increased more than 4-fold in males when compared both to control animals and their own baseline values. Females at 200 mg/kg/day appeared to have a trend towards elevated ALT ($\uparrow$ 75% vs. controls) but this was not statistically significant and the sample size was small ($n=4$ /sex/group). Triglycerides were elevated in males at 200 mg/kg/day but not in females.

Clear TAK-375 target organs were identified in all nonclinical species upon gross necropsy and histopathologic assessment of tissues and were relatively consistent:

Mouse

In female mice, the liver was unusually dark, visibly enlarged and exhibited an increase in weight with doses $\geq$ 300 mg/kg/day. Microscopic assessment revealed hepatocellular hypertrophy in the centrilobular region which was rated as slight to mild in severity. Biochemical analysis of tissue from necropsy samples indicated a significant increase in the activity of hepatic drug metabolizing enzyme aminopyrine N-demethylase relative to controls but no effect was noted on aniline hydroxylase. Other organs with treatment-related findings included the adrenal gland of both males and females in which vacuolization of cortical cells were noted at 1000 mg/kg/day. Vacuoles were observed in the zona fasciculata and in both sexes were considered fine and slight in nature though the adrenal gland in females also demonstrated evidence of hypertrophy. The ovary also appeared to be a target of TAK-375 in mice and demonstrated evidence of vacuolization of interstitial cells with a dose of 1500 mg/kg/day for 4-weeks but this was not observed at lower doses or in a 13-week study with maximum dose of 1000 mg/kg/day.

Rat

Rats also demonstrated TAK-375 alterations in liver morphology and histology. Liver weight was increased significantly at dose levels ≥ 150 mg/kg/day and centrilobular hepatocellular hypertrophy was evident with dosing ≥ 40 mg/kg/day in the chronic 26-week toxicity study. Analysis of hepatic enzyme activity from the 26-week study did not reveal a significant increase at 150 mg/kg/day though some reduction in plasma exposure to TAK-375 was noted in the study. Statistically significant increases in hepatic enzymes were noted, however, in 4-week toxicity studies in which a significant increase in aminopyrine N-demethylase activity, aniline hydroxylase was observed with dosing ≥ 150 mg/kg/day. Thyroid weight was increased with TAK-375 dosing of 4-weeks or longer with dosing ≥ 40 mg/kg/day in males and 150 mg/kg/day in females. The increase in weight was paralleled by findings of follicular cell hypertrophy. Assessment of thyroid hormones T_4 and T_3 as well as TSH indicated a reduction in T_3 and increase in circulating TSH with doses ≥ 150 mg/kg/day. T_4 was reduced only at a dose of 1500 mg/kg/day though an increase in UDP-glucuronosyltransferase enzyme activity for T_4 was noted in a 4-week rat toxicity study at a dose of 600 mg/kg/day. As in mice, the adrenal gland of rats increased in weight with exposure to TAK-375 and demonstrated a diffuse cortical vacuolization observed in females at dose levels ≥ 40 mg/kg/day and in males at 150 mg/kg/day. Also replicating the changes observed in mice, the ovaries of rats administered TAK-375 at doses ≥ 40 mg/kg/day exhibited vacuolization of interstitial cells. Other target organs included the kidney with increased weight without underlying histopathology; the spleen which was increased in weight in the 26-week study and demonstrated evidence of extramedullary hematopoiesis, considered related to the mild anemia displayed in this study.

Monkey

The primary target organ in monkeys administered TAK-375 up to 200 mg/kg/day in 4-week and 39-week toxicity studies was the liver. Liver weight was increased in the 4-week study at doses ≥ 200 mg/kg/day without evidence of an underlying histopathology though increased ALT was noted in males and females at 200 mg/kg/day. Samples of the liver were analyzed and demonstrated increased aminopyrine N-demethylase activity in a minority of animals with doses ≥ 12 mg/kg/day and in aniline hydroxylase activity with doses ≥ 50 mg/kg/day. In the 39-week study liver weight was increased at 200 mg/kg/day and under microscopic assessment of this tissue revealed diffuse hepatocellular hypertrophy that was not zonal in nature. Further indication that the increase in liver weight was not an adaptive response to enzyme induction were the observations that no enzyme induction was detected in contrast to the 4-week study and that ALT increases were marked in the males (> 4 -fold vs. controls) and elevated though this did not reach statistical significance in females.

The following table, adapted from the Sponsor, describes the exposure margins between the NOAEL in nonclinical studies and the expected therapeutic exposure to TAK-375 and metabolites with the use of Ramelteon at the indicated 8 mg dose.

Exposure to TAK-375 and Metabolites at the Nonclinical NOEL and the Margin of Exposure (MOE) over the Predicted Human Exposure* with Therapeutic Use of Ramelteon										
Species	Study Duration	NOAEL Dose (mg/kg/day)	TAK-375		M-II		M-III		M-IV	
			NOAEL AUC ₀₋₂₄ (ng·h/mL)	Human MOE	NOAEL AUC ₀₋₂₄ (ng·h/mL)	Human MOE	Human MOE	NOAEL AUC ₀₋₂₄ (ng·h/mL)	Human MOE	
Mouse	13-Weeks	100 [§]	8681	924	7143	30	253	9461	166	
Rat	26-Weeks	10	1943	207	1336	6	33	1344	27	
Rabbit	12-days [†]	60	22000	2340	4769 [§]	20	112	3354	59	
Monkey	39-Weeks	12	1626	173	12.6	0.05	6.3	14	0.25	
	4-Weeks [‡]	30			8696	36				

* Estimated from study 01-01-TL-375-003 from exposure in elderly to 16 mg Ramelteon and assuming linear kinetics. AUC₀₋₂₄ for TAK-375, M-I, M-II, M-III, and M-IV with a Ramelteon dose of 8 mg is estimated at 9.4, 8, 241.3, 3.5, and 57 ng·h/mL. Note that in the table above the M-II metabolite is next to TAK-375 to keep the active parent and metabolite together for easier reference.

† The Sponsor's NOAEL in the study report M-11-00178

‡ From the Segment II embryofetal development study in the rabbit.

§ From the 4-week repeat-dose toxicology study conducted with intravenous administration of M-II (25, 85) to cynomolgus monkey

¶ Estimated from toxicokinetic data in the rabbit at a dose of 300 mg/kg/day and assuming linear kinetics.

Genetic toxicology:

A standard battery of genetic toxicity tests comprising a bacterial reverse mutation assay, *in vitro* chromosomal aberration assay in CHL cells, and *in vivo* micronucleus assays in mice and rats were conducted with TAK-375. Several additional tests including an *in vitro* cell gene mutation test and an *in vivo, in vitro* unscheduled DNA synthesis assay in rat hepatocytes were subsequently conducted for further informational support. The Sponsor also performed follow-up studies to demonstrate assay exposure to TAK-375 metabolites when the parent compound was incubated with the S9 liver fraction containing rat hepatic microsomes. All studies with the exception of the *in vitro* chromosomal aberration were judged to be negative for evidence of genotoxicity. The *in vitro* chromosomal aberration study conducted in CHL cells demonstrated an increase in structural and numerical aberrations with exposure to TAK-375 in direct assays of 24 and 48 hr as well as a +S9 activation condition. This assay failed to provide an adequate evaluation of the clastogenic potential of TAK-375 in the -S9 activation condition due to the inappropriate use of a positive control which is known to require metabolic activation (i.e. +S9 condition). Thus, the lack of clastogenicity demonstrated by TAK-375-exposed cells in this condition cannot be assured due to the failure of the positive control. Although the weight of evidence suggests TAK-375 is not genotoxic, the Sponsor did not provide adequate data to explain the positive result obtained. As such, a genotoxic effect cannot be ruled out and should be included in the label as such unless a convincing explanation is brought forward by the Sponsor or the study is repeated without demonstration of clastogenic effects.

Carcinogenicity:

The tumorigenic potential of TAK-375 was assessed in B6C3F₁ mice as well as Sprague-Dawley rats in 2 year bioassays. TAK-375 doses used in the mouse study were 0, 30, 100, 300 or 1000 mg/kg/day by oral gavage. Dosage levels in the rat study were 0, 15, 60, 250 or 1000 mg/kg/day by oral gavage. Plasma concentrations of TAK-375 and metabolites M-I through M-IV were assessed on Day 1, Week 13 and Week 52.

In the mouse study, excessive mortality was observed at the 1000 mg/kg/day level which necessitated the early termination of this group at Week 94. The presumptive cause of death at this dose was the development of hepatic tumors. In surviving animals hepatic tumors were found in males and females treated with doses of ≥ 300 mg/kg/day. These

tumors consisted of hepatic adenomas, carcinomas and blastomas in males while in females adenoma was demonstrated with TAK-375 dose levels of ≥ 300 mg/kg/day and carcinoma was observed only in the 1000 mg/kg/day female group. Combining adenoma and carcinoma in males reduced the level at which a significant difference in tumors was found to 100 mg/kg/day, relative to control group animals. The NOAEL dose in male mice was 30 mg/kg/day and was associated with a plasma concentration which provides a 103-fold safety margin for TAK-375 compared with the expected human clinical exposure while for the active metabolite M-II the safety margin is 3-fold over the human exposure and the margin for M-IV is 64-fold. The NOAEL dose for tumors in female mice was 100 mg/kg/day which provides a safety margin of 827-fold for TAK-375 and 12-fold for M-II over the expected human therapeutic exposure while M-IV has a 105-fold margin. Evidence for hepatic enzyme induction was suggested in both species with marked reductions in plasma exposure with dose levels of ≥ 100 mg/kg/day in both males and females.

Administration of TAK-375 to rats for up to 104 weeks at dose levels of 0, 15, 60, 250 or 1000 mg/kg/day was associated with excessive mortality in the 1000 mg/kg/day group females which required termination of this group during Week 94. Early mortality with dose levels ≤ 250 mg/kg/day were ascribed to the development of pituitary tumors, while at 1000 mg/kg/day mortality was considered to be due to TAK-375-induced hepatic tumors or intoxication. The latter explanation was given for deaths early in the study period and occurring shortly after administration of the test article. TAK-375-induced hepatic tumors were found in males treated with ≥ 250 mg/kg/day (adenomas at ≥ 250 mg/kg/day, carcinomas at 1000 mg/kg/day) and in females treated with ≥ 60 mg/kg/day (adenomas ≥ 60 mg/kg/day, carcinomas at 1000 mg/kg/day). Administration of TAK-375 at 1000 mg/kg/day was also associated with the development of Leydig cell tumors in males. The rat liver tumor NOAEL was considered to be 60 mg/kg/day in males and 15 mg/kg/day in females. These doses resulted in plasma levels of TAK-375, M-II, and M-IV which in males provided a safety margin of 1,419-fold, 12-fold and 27-fold, respectively, over the expected human therapeutic exposure at 8 mg/day Ramelteon. In females the safety margin is considered to be 472-fold, 16-fold and 70-fold for TAK-375, M-II, and M-IV metabolites, respectively. Evidence for hepatic enzyme induction was noted for males and females in groups receiving ≥ 250 mg/kg/day TAK-375 based on the reduction in exposure to TAK-375 observed over the duration of the study.

Then tables below compare the NOAEL exposure to TAK-375 and M-II in the mouse (top table) and rat (bottom table) with comparison to the predicted human exposure at the therapeutic dose of Ramelteon as indicated by the Sponsor.

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Comparison of TAK-375 and M-II Exposures at the Mouse Tumor NOAEL versus the Expected Therapeutic Exposure with Ramelteon 8 mg/day in Humans

Tumor/Type	SEX	NOAEL DOSE (mg/kg/day)	TAK-375		M-II	
			AUC _{0-24h} (ng·h/mL)	HUMAN MOE†	AUC _{0-24h} (ng·h/mL)	HUMAN MOE†
LIVER						
Adenoma	M	100	10.138	1,079-fold	2,203	30-fold
	F	100	7,773	827-fold	2,783	32-fold
Carcinoma	M	100	10.138	1,079-fold	2,203	30-fold
	F	300	21,061	2,241-fold	7,996	33-fold
Adenoma + Carcinoma (combined)	M	30	967	103-fold	614	3-fold
	F	300	7,773	827-fold	2,783	32-fold
Hepatoblastoma	M	100	10.138	1,079-fold	720	30-fold
	F	1000	48,829	5,195-fold	14,956	> 60-fold

†. MOE = Margin of Exposure; based on results from study 01-01-TL-375-03; elderly administered 16 mg with an extrapolation to 8 mg based on linear kinetics within this dose range. Estimated AUC_{0-24h} TAK-375: 9.4 ng·h/mL; M-II: 241.3 ng·h/mL.

Comparison of TAK-375 and M-II Exposures at the Rat Tumor NOAEL versus the Expected Therapeutic Exposure with Ramelteon 8 mg/day in Humans

Tumor/Type	SEX	NOAEL DOSE (mg/kg/day)	TAK-375		M-II	
			AUC _{0-24h} (ng·h/mL)	HUMAN MOE†	AUC _{0-24h} (ng·h/mL)	HUMAN MOE†
LIVER						
Adenoma	M	60	13,432	1,419-fold	2,933	12-fold
	F	15	4,434	472-fold	3,773	16-fold
Carcinoma	M	250	42,933	4,472-fold	3,359	14-fold
	F	250	91,839	9,770-fold	22,278	92-fold
TESTIS						
Leydig cell tumor	M	250	42,933	4,472-fold	3,359	14-fold
	F			N/A		

†. MOS = Margin of Exposure; based on results from study 01-01-TL-375-03; elderly administered 16 mg with an extrapolation to 8 mg based on linear kinetics within this dose range. Estimated AUC_{0-24h} TAK-375: 9.4 ng·h/mL; M-II: 241.3 ng·h/mL.

Reproductive toxicology:

The effects of TAK-375 on reproductive potential as well as embryofetal development and pre- and postnatal development was studied in the Sprague-Dawley rat and Japanese White rabbit (the latter in the embryofetal study only).

Fertility and early embryonic development: Administration of TAK-375 by oral gavage at dose levels of 0, 6, 60, or 600 mg/kg/day to males 4 weeks prior to mating through mating and in females from 2 weeks prior to mating through Day 6 of pregnancy. Mortality was observed at the high dose which was unexpected due to previous experience with this dose in repeat-dose toxicology studies. Upon further study this was determined to be due an age effect such that 12-week old females in the fertility study had roughly twice the exposure as 6-week old females used in earlier studies. Assessment of surviving females in this group and the other TAK-375-treated groups indicated the following reproductive effects: females at a dose level ≥ 60 mg/kg/day demonstrated irregularity in the estrus cycle, reductions in the number of implants and reduction in the number of live embryos. The incidence of pre- and post-implantation loss was increased in the 60 mg/kg/day treatment group only and thus was considered to be incidental while a decrease in the number of corpora lutea was noted only at the 600 mg/kg/day dose level. No effects were observed in a supplemental study when TAK-

375 was administered in a 2nd mating to males and no evidence of an effect on sperm count, motility or morphology was noted. A supplemental study explored a different dose range in the Segment I study (20, 60 and 200 mg/kg/day). Only irregular estrus cycles were noted in the 60 mg/kg/day group; no difference in the number of implants or live embryos was shown as in the initial study. The Sponsor does not provide an explanation for this difference between the studies. The ages of the animals, dosing duration of females, and other potential variables were similar. Toxicokinetics were not obtained in the 2nd study therefore the extent or adequacy of exposure cannot be determined. For this reason the initial study was used for identification of NOAEL levels and risk assessment. The NOAEL for fertility and early embryonic development is considered to be 20 mg/kg/day as no effects were observed at this dose in either study. As toxicokinetic analysis was not completed for this study a body surface area conversion of the dose into mg/m² provides a margin of safety over the expected human exposure of 26-fold in a 65 kg individual.

Embryofetal development: Embryo-fetal development "Segment II" studies were conducted in the Sprague-Dawley rat and Japanese White rabbit. In the rat study, TAK-375 was administered at 10, 40, 150 or 600 mg/kg/day to pregnant 13-week old female rats from Day 6 – 17 of pregnancy with C-section on Day 20. Resulting maternal and fetal effects in TAK-375-treated groups were compared to vehicle-treated dams and their fetuses. Evidence of maternal toxicity was noted in dams treated with TAK-375 $\geq$ 150 mg/kg/day. Fetuses examined from TAK-375-treated groups revealed malformations including diaphragmatic hernia as well as increased number of lumbar vertebrae observed in fetuses from dams treated with $\geq$ 150 mg/kg/day. Variations in the 150 mg/kg/day group consisted of skeletal findings such as irregular lumbar rib shape and scapula. At 600 mg/kg/day malformations observed included cysts on the external genitalia in addition to the increased incidence of diaphragmatic hernia and increased lumbar rib number noted in fetuses of the 150 mg/kg/day group. Variations in this group resembled those in the 150 mg/kg/day group with the additional observation of split sternbra. Therefore, the maternal, embryo-fetal, and teratogenic NOAEL are considered to be 40 mg/kg/day. Subsequent examination of the M-II metabolite in the rat involved intravenous administration of up to 30 mg/kg M-II to pregnant female rats over Days 6-17 of gestation. No maternal toxicity was noted which includes no observed effect on measures of fertility. Administration of M-II at a dose of 30 mg/kg/day i.v. also did not alter fetal body weight, sex ratio, % alive at C-section or the incidence of external, visceral or skeletal abnormalities when compared to control litters.

The Segment II study conducted in the Japanese White rabbit involved administration of TAK-375 at doses of 0, 12, 60 or 300 mg/kg/day by oral gavage to pregnant females over Days 6-18 of pregnancy. No adverse effects were noted with dose levels of $\leq$ 60 mg/kg/day. TAK-375 at 300 mg/kg/day produced clinical signs of toxicity and transient reduction in body weight gain without evidence of effects on the fetus including number alive at C-section, body weight, sex ratio, or the incidence of external, visceral or skeletal abnormalities and variations upon examination. An apparent increase in the observation of irregular lumbar rib was within historical control data ranges for this lab for which data was provided. The maternal NOAEL was identified at 60 mg/kg/day while the embryofetal teratogenicity NOAEL in the rabbit was $>$ 300 mg/kg/day.

Pre- and postnatal development: A Segment III study of pre- and postnatal development was conducted using Sprague-Dawley rats. TAK-375 was administered by oral gavage to pregnant dams (F₀ generation) from Day 6 of pregnancy through parturition to Day

6 of lactation at doses of 0 (vehicle), 30, 100 or 300 mg/kg/day. In F₀ dams treated with TAK-375 or vehicle, no toxicities were observed with dosing at 30 mg/kg/day while at TAK-375 ≥ 100 mg/kg/day increased adrenal gland weight was found at necropsy and was also associated with occasional observations of reduced body weight and body weight gain during the lactation period with the 300 mg/kg/day dose reducing body weight gain during pregnancy. No effects on reproductive performance were observed in F₀ dams with TAK-375 dosing up to 300 mg/kg/day, however. F₁ offspring in the 30 mg/kg/day group did not demonstrate abnormalities on any parameter. F₁ offspring in the 100 mg/kg/day litters had reduced body weight during weaning as well as after weaning compared with their control litter offspring. This reduction in body weight was more prominent in 300 mg/kg/day F₁ offspring which also had evidence of developmental delays including an increased latency to eruption of lower incisors, reduced rate of acquisition of the righting reflex, and altered emotional responses when compared to their control F₁ litter counterparts. Also observed in the F₁ offspring in the 300 mg/kg/day litters only were an increased incidence of diaphragmatic hernia. F₁ offspring selected for evaluation of reproductive potential by mating and generation of the F₂ generation did not find an effect of TAK-375 exposure on reproductive performance when compared with F₁ offspring in the vehicle-treated control group. No differences were seen in the F₂ generation between treatment groups. The NOAEL for maternal toxicity in the F₀ dams was identified at 30 mg/kg/day due to clinical observations of reduced body weight and food consumption as well as increased adrenal gland weight with dosing of 100 mg/kg/day. For the F₁ generation the NOAEL (developmental toxicity) was 30 mg/kg/day due to observations of body weight in the 100 mg/kg/day litters which extended to beyond the lactation period while at the highest dose level, 300 mg/kg/day, developmental delays and alterations in behavior could be observed as well as an increased incidence of diaphragmatic hernia.

The following table summarizes the margins of safety or exposure margin for the maximum daily human dose of 8 mg Ramelteon, compared to the nonclinical NOAEL dose or exposure in the reproductive toxicology studies:

Human Margins of Safety or Exposure over the Nonclinical NOAEL in Reproductive Toxicology Studies						
Study	Species	NOAEL Type	NOAEL Dose (mg/kg/day)	Margin of Safety or Margin of Exposure*		
				BSA [‡]	AUC _{0-24h}	
				TAK-375	M-II	
Segment I	Rat	Fertility and early embryonic development	20	26-fold	n.d.	
Segment II	Rat	Maternal	40		1,892-fold	45-fold
		Embryofetal				
	Rabbit	Maternal	60	157-fold	2,340-fold [†]	n.d.
		Embryofetal	300		11,862-fold [†]	99-fold [‡]
Segment III	Rat	F ₀ (maternal) F ₁ development	30	39-fold	n.d.	

* Estimated from study 01-01-TL-375-003 from exposure in elderly to 16 mg Ramelteon and assuming linear kinetics. AUC_{0-24h} for TAK-375, M-I, M-II, M-III, and M-IV with a Ramelteon dose of 8 mg is estimated at 9.4, 8.2, 11.3, 3.3, 5, and 57 ng·h/ml.

[‡] Body surface area conversion based on a 65 kg individual

n.d., not determined

[†] Based on toxicokinetic data from the original Segment II study (M-11-001003.001)

[‡] Based on data from a follow-up toxicokinetic study in non-pregnant 13 week old female rats

B. Pharmacologic activity

TAK-375 and its active metabolite M-II are high affinity, high potency full agonists the melatonin-1 (ML₁) receptors. Melatonin receptors have been classified by molecular and pharmacological analysis into 3 subtypes: high-affinity MT1 and MT2 receptors (K_d = 30-150 pM) which were formerly classified as subtypes of the ML1 receptor (ML_{1A} or ML_{1B}, respectively; also mel_{1a} and mel_{1b}, respectively), and lower-affinity MT3 receptors which exhibit nanomolar affinity for melatonin [see figure from Dubocovich et al., 1999, below].

	mt ₁	mt ₂	MT ₃
Nomenclature			
Other Names	Mel _{1a} , ML _{1A}	Mel _{1b} , ML _{1B}	ML ₂
Affinity (K _D)	45 pM	149 pM	0.3-2 nM
Pharmacology	MLT >> NAS	MLT >> NAS	MLT > NAS
Selective Agonists	---	---	N-Acetyl 5HT 5-MCA-NAT
Selective Antagonists	---	4P-ADOT 4P-PDOT	PRAZOSIN

Figure 1. Nomenclature for mammalian melatonin receptors. The nomenclature described here was adopted by the Nomenclature Committee of International Union of Pharmacology in 1998. For further details please see reference 25. Luzinole: MLT: melatonin (N-acetyl-5-methoxytryptamine); NA: 5-HT; N-acetyl-5-hydroxytryptamine; 5-MCA-NAT: 5-methoxy-carbonylamino- N-acetyltryptamine; 4-P-PDOT: 4-phenyl 2-propionamidotetrahydroquinoline; 4-P-ADOT: 4-phenyl 2-acetamidotetrahydroquinoline.

The mt1 and MT2 receptors have been cloned and demonstrate 60% homology between each other and are members of the G-protein-linked 7-transmembrane superfamily. Functionally these receptors have been shown to inhibit cAMP production through pertussis-sensitive Gi protein inhibition of adenylate cyclase. A parallel stimulation of PGF2 α leading to PLC β activation has also been demonstrated resulting from release of the $\beta\gamma$ -subunits of the G_i-protein. The MT3 receptor is believed to be a melatonin-sensitive quinone reductase 2 enzyme and will not be discussed further as TAK-375 was not studied for interaction with this melatonin-sensitive enzyme.

Melatonin receptors are found distributed throughout nearly all tissues of the body. Animal studies investigating 2-[¹²⁵I]-iodomelatonin-binding revealed significant binding of this specific melatonin receptor agonist in the pars tuberalis of the adenohypophysis, superior colliculus and neural retina (thought involved in visual function), SCN, arteries, and reproductive tissues including the epididymis in the male, granulosa of preovulatory follicles, and prostate epithelium.

Binding studies in cell lines transfected with melatonin receptor subtypes as well as in tissue homogenates expressing ML₁ (i.e. MT1 and MT2 receptors) or ML₂ (i.e. MT3) receptors indicated TAK-375 has picomolar affinity for MT1 and MT2 receptors. TAK-375 binding affinity (K_d) for MT1, MT2 and MT3 receptors was estimated at 11.6 - 14 pM, 112 pM, and 2.65 μ M, respectively. TAK-375 therefore shows a 25,000 - 190,000-fold binding

differential between the ML₁ receptor subtypes and the ML₂ (MT3) receptor subtype. The M-II metabolite of TAK-375 exhibits a K_d ~ 114 pM at the MT1 receptor and 566 pM at the MT₂ receptor. TAK-375 therefore demonstrates a 5-10-fold higher affinity for the ML₁ receptor subtypes when compared with its metabolite M-II. Functional studies using TAK-375 and M-II revealed picomolar inhibition of forskolin-stimulated cAMP accumulation with the TAK-375 having approximately 17 – 25-fold higher potency than the metabolite M-II. Both TAK-375 and M-II demonstrated little affinity or efficacy at non-ML₁ receptor sites when tested in a broad screen of receptors and enzymes though M-II did demonstrate inhibition of 5-HT_{2B} receptor function at with a K_i ~ 1.7 μM, representing 1000-fold lower potency than M-II at the MT1 receptor.

C. Nonclinical safety issues relevant to clinical use

Mutagenicity:

Although the overall weight of evidence from *in vitro* and *in vivo* genetic toxicology assays suggest TAK-375 and TAK-375 metabolites are not genotoxic, the positive finding of clastogenicity in the *in vitro* chromosomal aberration assay in Chinese hamster lung cells cannot be dismissed without further investigation or convincing explanation from the Sponsor.

Hepatic adenoma, carcinoma and hepatoblastoma:

TAK-375-treatment of both mice and rats resulted in consistent development of hepatic tumors implies a cross-species mechanism which merits potentially greater concern. The Sponsor concluded that based on the evidence from repeat-dose toxicology studies in both mice and rats. These results were further supported by a dedicated one-week repeated-dose oral administration study of TAK-375 to mice and rats in which a broad panel of substrates for specific cytochrome P450 enzymes was screened and indicated significant induction. Drug-induced induction of hepatic drug-metabolizing enzymes has been suggested and generally accepted as the mechanism behind phenobarbital-induced hepatocellular adenoma and carcinoma in mice and rats (Whysner et al., 1996). TAK-375 administration for 1-week to mice and rats produced a pattern and magnitude of enzyme induction which paralleled that of phenobarbital. According to the current opinion of the scientific community, the development of hepatic tumors in rodents is likely due to the induction of hepatic microsomal enzymes; however, this mechanism is not thought to be operative in humans and serves to mitigate this reviewer's concern.

The Sponsor has proposed an alternative explanation for the hepatic tumor findings, involving a mechanism by which TAK-375 may increase dehydroepiandrosterone (DHEA) levels as melatonin has been demonstrated to stimulate DHEA levels both *in vitro* and *in vivo*. DHEA has been reported to be a peroxisome proliferator therefore may lead to the development of tumors through this route though it has also been shown to induce hepatic microsomal enzymes. Notably however, no evidence of peroxisome proliferation was found from histopathologic reports. Further evidence this mechanism is unlikely to be involved was a dedicated 4-week repeat-dose study designed to determine the effect of TAK-375 on plasma melatonin and DHEA levels and to correlate this to ultrastructural changes in hepatocytes using dose levels identical to the rat study. TAK-375 administration produced

an elevation in circulating melatonin and DHEA levels but on microscopic examination the changes in hepatocytes were limited to expansion of the smooth endoplasmic reticulum consistent with hepatic enzyme induction and no evidence of peroxisome proliferation was noted. Additionally, the activity of acyl-CoA oxidase, a marker for peroxisome proliferation, was not increased at any dose level. Taken as a whole, the evidence appears to support the hypothesis that the hepatic tumors observed in rodents are a consequence of significant induction of hepatic microsomal enzymes. This mechanism is unlikely to lead to tumors in humans as the clinical experience with hepatic enzyme inducing drugs such as phenobarbital suggest a very low order of risk. Nevertheless, as some evidence of genetic toxicity was present as described above, a genotoxic mechanism for carcinogenesis cannot be ruled out.

Leydig cell tumor:

The development of Leydig cell tumors in the rat was proposed by the Sponsor to be a result of inhibition of testosterone by TAK-375 and a consequent release from feedback inhibition on LH release from the pituitary resulting in overstimulation of the Leydig cells and development of hyperplasia and adenoma. This mechanism is considered plausible as compounds which interfere with testosterone synthesis, are GnRH agonists, or promote release of GnRH are associated with an increase in LH levels and the development of this tumor. Notably, demonstration of this mechanism would not be considered to confer great risk to humans as the sensitivity of human Leydig cells to LH has been shown to be much weaker than the rat (Cook et al., 1999). The Sponsor provided evidence that daily oral administration of TAK-375 for 1-week or 4-weeks to rats produced a suppression of testosterone synthesis at dose levels at which were associated with Leydig cell hyperplasia and adenoma in the 2-year bioassay. Weaker evidence, however, was demonstrated for an elevation in circulating LH levels. Though an elevated LH was found in the 4-week study using a large sample size, no evidence was found in the 1-week study and the time-points at which LH differed significantly from control animals in the 4-week study were only at 4.5 hr (and not at 4 hr or 5 hr) and at 24 hr after the final administration of TAK-375. Thus, the Sponsor's proposed mechanism is supported by some data; however, definitive evidence for this explanation is, in this reviewer's opinion, lacking. Nevertheless, the development of this tumor in the rat occurs with an exposure level which affords a large margin of safety for human use. This margin is not as comforting if data from further genotoxicity assays do not refute TAK-375 as a clastogenic genotoxicant.

Thyroid hypertrophy:

Toxicologic observations in the thyroid of nonclinical species were limited to increased thyroid weight with histologic evidence of follicular cell hypertrophy in the rat only. Thyroid hormones were altered in the rat with repeated administration of TAK-375 in all studies of 4-weeks duration or greater. Changes typically consisted of a depression of T_3 and elevation in TSH, the latter hormone up to 3 – 8-fold control values in a 13-week study in the rat. Alterations in serum T_4 were not observed in any study, however. The Sponsor demonstrated an increase in UDP-glucuronosyltransferase activity involved in T_4 metabolism which supports a documented cascade of microsomal enzyme induction producing increased glucuronidation and subsequent excretion of T_4 which causes a transient reduction in circulating T_4 levels which removes feedback inhibition on TSH release from the pituitary and the increased TSH levels lead to both restores the proper level

of circulating thyroid hormone level (principally T₄) and follicular cell hypertrophy. If this increase in microsomal enzyme activity is long term the excessive stimulation of the thyroid by TSH is believed to promote thyroid tumors (Vriend, 1983; Hood et al., 1999; Hood et al., 2003). Though several elements of this cascade generally seem in play (i.e. microsomal enzyme activation involving metabolism of a thyroid hormone, increased TSH levels, etc.) the levels of T₃ are observed to be reduced in circulation, not T₄, and the Sponsor has not demonstrated that the hepatic enzymes involved in T₃ glucuronidation and elimination are elevated in activity. There are several published studies which have demonstrated an induction of microsomal enzymes causing a reduction in T₄ and compensatory TSH increase without evidence of a change in T₃ levels such as was noted in the current studies (Liu et al., 1995; Hood et al., 2003). A reduced serum T₃ without a similar change in T₄ and consequent increase in TSH production could be effected by interference with thyroid hormone metabolism, specifically the 5'-monodeiodinase which is responsible for the conversion of T₄ → T₃ (Capen, 1997). Evidence also exists that in rat suggesting that negative feedback on pituitary TSH secretion is principally mediated by T₃ (Emerson et al., 1989). On the other hand, melatonin has recently been suggested to inhibit thyroid function through its anti-oxidant properties; reactive oxygen species and free radicals such as H₂O₂ are involved in thyroid hormone production (Karbownik and Lewinski, 2003) and therefore a direct effect of a melatonin receptor agonist such as TAK-375 on thyroid function cannot be ruled out.

No evidence for thyroid changes was observed in the mouse or monkey on gross or microscopic examination of this tissue, however. Although hepatic enzyme induction in the monkey was limited at best, significant microsomal induction was observed in the mouse without evidence of thyroid effects which leads this reviewer to question whether this finding may also then be specific to the rat. Irrespective of the mechanism involved, the rat is known to be highly sensitive to the development of thyroid pathology as this species lacks thyroxine-binding globulin (TBG), the high affinity plasma protein which carries 70% of human T₄ and T₃, and instead relies upon the lower affinity transthyretin and albumin as a buffer for alterations in thyroid hormone concentrations. This reduced buffering capacity in the rat model causes greater fluctuations in free T₄ and T₃ and therefore a greater sensitivity for TSH release when levels of these hormones are suppressed. An additional aspect which mitigates concern is that the human is less dependent on TBG for appropriate regulation of thyroid hormone levels and TSH concentrations as several mutations in the coding region of the TBG gene have been documented to result in the absence of this protein in the human (Ueta et al., 1997; Miura et al., 2000) and is considered to be of little clinical concern (Barlow, 1997) as appropriate free T₃, T₄, and circulating TSH levels are maintained though total levels of T₃ and T₄ are reduced.

The toxicological significance of the observation of thyroid hypertrophy in the rat for the determination of human risk is not clear. Of note, in rats the increased thyroid weight resolved with a 13-week treatment-free period suggesting the disruption produced is not permanent. Also notably, the 104-week carcinogenicity bioassays did not reveal a treatment-dependent increase in thyroid tumors in either mouse or rat. On balance, the concern over significant thyroid toxicity in the human based on the nonclinical data is low.

Adrenal and ovarian vacuolization:

Adrenal gland. Studies in rodents consistently detected the development of adrenocortical vacuolization characterized as slight to mild in severity without necrosis and coincident with increased adrenal gland weight. This histopathologic change was observed to begin at the lower end of the dosing ranges used in rat studies though mice demonstrated this at higher doses and also showed evidence at the upper range of adrenal hypertrophy as well. In a rat study which contained both 4-week and 13-week recovery groups after 4-weeks of TAK-375 treatment this increase in adrenal weight and the adrenocortical vacuolization was still noted at 4-weeks after TAK-375 cessation but neither increased adrenal weight or vacuolization were present with a 13-week treatment-free period suggesting this finding is reversible. Adrenocortical vacuolization has been previously demonstrated to occur with inhibition of ACTH release, disrupted steroidogenesis and impairment in release of glucocorticoids from the adrenal gland (Rosol, 2001; Capen, 2001). Melatonin itself has been shown to inhibit corticosterone release into circulation as well as reduce the pituitary response to corticotrophin releasing factor resulting in a decline in ACTH levels in circulation (Preslock, 1984). It is believed likely by the Sponsor that TAK-375 is causing this response in the adrenal gland by a similar direct pharmacologic action exerted through melatonin receptors in the pars tuberalis and pituitary as well as in the adrenal gland. The Sponsor did not, however, characterize the nature of the vacuoles observed to determine if they were consistent with lipid accumulation via inhibition of steroidogenesis or the more generalized phospholipidosis, though evidence from the latter was not found with liver examination. The Sponsor did perform a dedicated study to examine the effects of TAK-375 administration for 1-week on the levels of circulating hormones from the pituitary and endocrine organs. Results indicated a trend towards reduction in ACTH levels when compared with controls though this change was not statistically significant. This reviewer considers the mechanism described by the Sponsor to be possible as the repeat-dose toxicology studies in which the adrenal gland findings were noted were of greater duration.

The adrenal gland of cynomolgus monkeys were not noted to exhibit overt or occult pathology with studies of greater than 4-weeks duration. Adrenal gland weight and structure were unaffected by treatment with TAK-375 for 4-weeks or 39-weeks though an increase in liver weight and adrenocortical hypertrophy characterized by decreased lipid in zona fasciculata cells was noted along with atrophy of the zona glomerulosa cells in monkeys used for dose range-finding studies of 2-weeks duration. It is not clear why these findings were not observed in the longer studies with oral TAK-375 administration at an identical dose level. The Sponsor does not provide an explanation as to this difference though physiologic or pharmacologic tolerance must be suspected. Studies with intravenous M-II did not detect an effect of this active metabolite on any parameter or organ including the adrenal gland in a 4-week study.

Although there is great variability between species in adrenal response to xenobiotics (Rosol, 2001) due to differences in metabolic pathways for steroidogenesis, it appears this finding should be considered to be a potential risk with human exposure to TAK-375 as the Sponsor did not elucidate the mechanism by which this change occurs and address its relevance to humans. However, NOAEL exposures in rodent are multiples of the exposure predicted in humans with therapeutic administration of Ramelteon. Also mitigating concern

is the finding that the adrenocortical vacuolization in the rat demonstrates evidence of reversibility with cessation of TAK-375 administration.

Ovary. A similar demonstration of interstitial cell vacuolization was noted in the ovaries of rodents administered TAK-375. Rats appeared more sensitive to this effect when compared with mice. This too reversed in rats allowed to complete a 4-week or 13-week recovery period after a 4-week administration of TAK-375. A similar explanation as to the adrenal gland previously may be offered: melatonin has been shown to inhibit the release of gonadotropins from the pituitary (Preslock, 1984) and is the basis for seasonality in reproduction observed in species known to be seasonal breeders (Malpoux et al., 1999). This effect is not considered to be of primary importance in the human though this may be due to manipulation of our environmental cues (Wehr, 2001) and could become of functional significance with exogenous stimulation of melatonin receptors. Though this concern is mitigated somewhat by the observation that ovarian vacuolization was not noted in the 4-week or 39-week monkey studies, this toxicity may not be species specific and could potentially occur with human exposure. A significant exposure margin does exist, however, between human therapeutic levels and the TAK-375 and M-II exposures in rodents associated with the NOAEL and this, along with the evidence of reversibility in the rodent, are the primary arguments for a lowered level of concern.

Use in pregnancy:

Teratogenicity. Administration of TAK-375 to pregnant rats during the critical period of organogenesis was associated with a dose-dependent observation of fetal abnormalities including diaphragmatic hernia and cysts on the external genitalia. External cysts were found on 3 fetuses in one litter while diaphragmatic hernia was observed in 13 fetuses from 6 litters. An increased incidence of skeletal variations including irregularly shaped scapula and ribs was also noted to occur with increasing dose of TAK-375. These malformations and variations occurred at a dose level which was associated with maternal toxicity but while the variations described are commonly seen in the setting of maternal toxicity, diaphragmatic hernia and the development of cysts on the external genitalia are not expected secondary to maternal effects. The clinical concerns are reduced by the safety margin conferred by the high rat maternal exposure at the embryofetal NOAEL. Based upon these findings, TAK-375 should be labeled a Pregnancy Category C drug and the details of the findings incorporated into the label.

Pre- and postnatal development. Maternal exposure to TAK-375 during the periods of implantation through postnatal weaning of offspring in the rat was associated with reduced postnatal weights which were noted both during and after weaning as well as delays or alterations in several measures of postnatal development including delayed eruption of lower incisors, delayed acquisition of the righting reflex, and emotional alterations. Although an assessment of emotionality was not conducted at a later time-point to assess the durability of the effect, both lower incisors and the righting reflex were fully acquired upon further assessment. Mating behaviors, as well as reproductive function, were not noted to be different from offspring of vehicle-treated dams, the former observation perhaps indicating no major change in emotional state in the long-term. Perhaps equally important, the maternal exposure at the pre- and postnatal NOAEL would be estimated to provide a comfortable

safety margin based on body surface area conversion. That the developmental delays appear likely to be transient also reduces concern for significant human risk.

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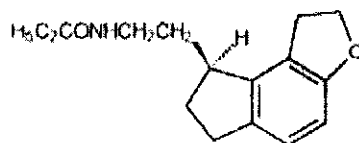
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-782
Review number: 1
Sequence number/date/type of submission: N000/September 22, 2004/Original
Information to sponsor: Yes (X) No ()
Sponsor and/or agent: Takeda Global
Manufacturer for drug substance: Takeda Pharmaceutical Company Limited
 Pharmaceutical Production Division
 4720, Takeda Mitsui
 Hikari, Yamaguchi
 Japan 743-8502

Reviewer name: Adam M. Wasserman, Ph.D.
Division name: Anesthesia, Analgesia, and Rheumatology Products
HFD #: 170
Review completion date: June 22, 2005

Drug:
Trade name: To Be Determined
Generic name: Ramelteon
Code name: TAK-375
Chemical name: (S)-N-[2-(1,6,7,8-Tetrahydro-2H-indeno[5,4- β]furan-8-yl)ethyl]propionamide
CAS registry number: 196597-26-9
Molecular formula; molecular weight: C₁₆H₂₁NO₂; 259.34
Structure:



Relevant INDs/NDAs/DMFs: IND 58,136; DMF L 3
Drug class: Melatonin receptor agonist
Intended clinical population: Treatment of insomnia

Clinical formulation: Each tablet contains 8 mg of Ramelteon along with the following inactive ingredients: lactose monohydrate, starch, hydroxypropyl cellulose, magnesium stearate,

hypromellose, copovidone, titanium dioxide, yellow ferric oxide, polyethylene glycol 8000 and ink containing shellac and synthetic iron oxide black. See Sponsor's table below.

Table 1 Composition of Ramelteon Tablets, 8 mg

Component	Reference to Quality Standards	Function	Quantity per Tablet (mg)
Core Tablet (uncoated tablet)			
Ramelteon	In-house standard	Active ingredient	8.0
Lactose monohydrate	NF	/	/
Starch	NF		
Hydroxypropyl cellulose	NF		
Magnesium stearate	NF		
Purified water	USP		
<i>Core tablet weight</i>			130.0
Printing Ink Solution			
Hypromellose	USP	/	/
Copovidone			
Titanium dioxide	USP		
Ferric oxide, yellow	NF		
Purified water	USP		
<i>Total tablet weight</i>			135.0
Polyethylene glycol 8000	NF	/	/
Purified water	USP		
Printing Ink Solution			
Printing Ink	Manufacturer's standard	Identification	(4)

[

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There are no new excipients in the proposed drug product.

The Sponsor's specifications for the Ramelteon drug substance is in the following table taken directly from the submission:

Table 1 Specification of Ramelteon Drug Substance

Test	Analytical Procedure	Acceptance Criteria

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Route of administration: Oral

Proposed dose: 8 mg/day

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

Studies reviewed within this submission:

Studies reviewed for this NDA:

Note: Studies listed in *italics* were examined for their accuracy and consistency and were summarized for the NDA but were not formally reviewed. Studies preceded by the symbol † were reviewed by Aisar Atrakchi, Ph.D. [HFD-120] for the IND. All other studies were formally reviewed.

Study Title	Document	Section/Title/Description
Pharmacology Study Reports		
<i>Melatonin Receptor Binding Profile of TAK-375</i>	M-11-00617	4.2.1.1 Primary Pharmacodynamics
<i>Effects of TAK-375 and M-II (2S, 8S) on Human MT2 Receptor Binding</i>	M-11-00616.001R	
<i>Melatonin Receptor Binding Profile of TAK-375 Metabolites</i>	M-11-00388.001R	

Study Title	Study ID	Section/Description
<i>Effects of TAK-375 on cAMP Production in the Rat Pituitary</i>	M-11-00619	
<i>Sleep Promotion Studies in Freely Moving Monkeys</i>	M-11-00572	
<i>Sleep-Promoting Action of M-II, Main Active Metabolite of TAK-375, in Monkeys</i>	M-11-00736	
<i>The Sleep Promoting Action of TAK-375 in Freely Moving Cats</i>	M-11-00613	
<i>Sleep Promoting Action of M-II (2S, 8S), A Metabolite of TAK-375, in Freely Moving Cats</i>	M-11-00624	
<i>Effects of TAK-375 on Restraint of Activity after a Phase Shift in Rats</i>	M-11-00323.001R	4.2.1.2 Secondary Pharmacodynamics
<i>Effects of TAK-375 on Learning and Memory in Rats</i>	M-11-00620	
<i>Effects of TAK-375 on Nocturnal Plasma Melatonin Levels in Rats</i>	M-11-00623	
<i>Comparison of Emetic Potentials Between TAK-375 and Melatonin in Monkeys</i>	M-11-00708	
<i>General Pharmacological Studies on TAK-375</i>	M-11-00106.001A	
<i>Effect of TAK-375 on Action Potential Parameters in Sheep Isolated Cardiac Purkinje Fibers</i>	M-11-00102	4.2.1.3 Safety Pharmacology
<i>Safety Pharmacology Studies of TAK-375 – Effects on hERG Current</i>	M-11-00557	
<i>Safety Pharmacology Studies of TAK-375 – Effects of M-II (2S, 8S) on hERG Current</i>	M-11-00562	
<i>Effects of TAK-375 on Body Temperature, Heart Rate, Mean Blood Pressure and Activity in Monkeys</i>	M-11-00095	
<i>Effects of TAK-375 and Melatonin on Various Receptors and Enzymes</i>	M-11-00618	
<i>Effects of M-II (2S, 8S), A Metabolite of TAK-375, on Various Receptors and Enzymes</i>	M-11-00389	
<i>Physical Dependency Study of TAK-375 in Rats</i>	M-11-0028.001	
<i>The Primary Physical Dependence Effects of TAK-375 in Rhesus Monkeys</i>	01-02-TL-375-013	
<i>Effects of TAK-375, Melatonin, and Benzodiazepine Agonists on Acquisition of a Conditioned Place Preference Task in Rats</i>	M-11-00621.001R	
<i>Behavioral Effects of TAK-375 and Self-Administration Studies in Monkeys</i>	01-02-TL-375-010	
<i>A Dependence Study of TAK-375 on Acute CNS Effects by Gross Behavioral Observations and Reinforcing Effect by Intravenous Self-Administration Experiment in Rhesus Monkeys</i>	M-11-00626	
<i>Response Discrimination of TAK-375 and Midazolam by Rhesus Monkeys</i>	01-02-TL-375-011	
<i>The Discriminative Stimulus Effects of TAK-</i>	01-02-TL-375-012	

		Discussion
<i>375 in Rhesus Monkeys: Possible Benzodiazepine Agonist-Like Effects in the Context of Precipitated and Drug Withdrawal Signs</i>		
<i>Effects of TAK-375 on Rota-Rod Performance in Mice</i>	M-11-00622	4.2.1.4 Pharmacodynamic Drug Interactions
Pharmacokinetics Study Reports		
<i>Absorption Site Of [14C] TAK-375 In Rats</i>	M-11-00748	4.2.2.2 Absorption
<i>Portal Absorption Of [14C] TAK-375 In Rats</i>	M-11-00749	
<i>Plasma Concentration Of Radioactivity After A Single Administration Of [14C] TAK-375 In Rats</i>	M-11-00778	
<i>Plasma Concentration Of Radioactivity During And After Repeated Oral Administration Of [14C]TAK-375 In Rats</i>	M-11-00780	
<i>Plasma Concentration Of TAK-375 And Its Metabolites M-II After A Single Oral Or Intravenous Administration Of TAK-375 In Rats</i>	M-11-00781	
<i>Concentration Of TAK-375 And Its Metabolites M-I, M-II, M-III And M-IV In Plasma After Oral Administration To Dogs</i>	M-11-00642	
<i>Concentration Of TAK-375 In Plasma After Oral Administration At Low Doses In Monkeys</i>	M-11-00386	
<i>Plasma Concentration And Urinary And Fecal Excretion After A Single Administration Of [14C] TAK-375 In Monkeys</i>	M-11-00779	
<i>Plasma Concentration Of TAK-375 And Its Metabolite M-II After A Single Oral Or Intravenous Administration Of TAK-375 In Monkeys</i>	M-11-00782	
<i>Permeability Study Of TAK-375 Using Caco-2 Cell Monolayers</i>	M-11-00750	
<i>Whole Body Autoradiography After A Single Oral Administration Of [14C] TAK-375 In Male And Female Rats</i>	M-11-00747	4.2.2.3 Distribution
<i>Plasma And Brain Concentrations Of TAK-375 And Its Metabolite M-II After A Single Oral Administration Of TAK-375 In Rats</i>	M-11-00783	
<i>Concentration of M-II and M-IV in Plasma and Brain after Oral Administration of TAK-375 to Rats.</i>	M-11-00640	
<i>Pigmented Eyeball Distribution</i>	M-11-00641	
<i>Placental Transfer Study</i>	M-11-00643	
<i>Pharmacokinetics of TAK-375 in Animals (absorption)</i>	M11-00049.001A	4.2.2.4 Metabolism
<i>The Half-life of Radioactivity in Tissues after Oral Administration of [14C] TAK-375 to Rats</i>	M-11-00847	
<i>Identification Of TAK-375 Metabolites In Rats And Monkeys</i>	M-11-00050	

Table 1: Description of Studies		
<i>Characterization Of TAK-375 Metabolites</i>	M-11-00752	
<i>Metabolic Profile Of TAK-375 In Portal Vein Plasma After Administration Of [14C] TAK-375 Into The Jejunal Loop Of A Rat</i>	M-11-00753	
<i>Metabolic Profile Of Plasma and Brain After A Single Oral Administration Of [14C] TAK-375 In Rats In Rat</i>	M-11-00784	
<i>The Metabolite Profiles In The Urine And Feces After A Single Oral Administration Of [14C] TAK-375 In Rats</i>	M-11-00785	
<i>The Metabolic Profiles In The Bile After A Single Intraduodenal Administration Of [14C] TAK-375 In Rats</i>	M-11-00786	
<i>Metabolic Profile Of Milk And Plasma After Oral Administration Of [14C] TAK-375 To Lactating Rat</i>	M-11-00754	
<i>Metabolic Profile Of Maternal And Fetal Plasma After Oral Administration Of [14C] TAK-375 To Pregnant Rat</i>	M-11-00755	
<i>The Metabolite Profiles In The Plasma After A Single Oral Administration Of [14C] TAK-375 In Monkeys</i>	M-11-00787	
<i>The Metabolite Profiles In The Urine And Feces After A Single Oral Administration Of [14C] TAK-375 In Monkeys</i>	M-11-00788	
<i>Effects Of TAK-375 On Hepatic Drug-Metabolizing Enzymes In Mice</i>	M-11-00594	
<i>Effects Of TAK-375 On Hepatic Drug-Metabolizing Enzymes In Rats</i>	M-11-00593	
<i>Milk Secretion Study</i>	M-11-00644	
<i>Urinary, Fecal And Expiratory Excretion After A Single Administration Of [14C]TAK-375 In Rats</i>	M-11-00789	4.2.2.5 Excretion
<i>Biliary Excretion And Enterohepatic Circulation After A Single Administration Of [14C]TAK-375 In Rats</i>	M-11-00790	
<i>Effect of TAK-375 and its Metabolites on the In Vitro Metabolism of Melatonin in Rats</i>	M-11-00649	
Toxicology		
<i>Oral Single Dose Toxicity Study Of TAK-375 In Rats</i>	M-11-00016	4.2.3.1 Single-Dose Toxicity
<i>Intravenous Single Dose Toxicity Study Of TAK-375 In Rats</i>	M-11-00078	
<i>Intravenous Single Dose Toxicity Study Of TAK-375 In Rats (Supplemental Study)</i>	M-11-00347	
<i>Oral Gavage Escalating Dose Toxicity Study Of TAK-375 In Monkeys</i>	M-11-00011	
<i>Preliminary Two-Week Oral Gavage Toxicity Study Of TAK-375 In Rats</i>	M-11-00610	4.2.3.2 Repeat-Dose Toxicity
<i>Preliminary Two-Week Oral Gavage Toxicity Study Of TAK-375 In Rats (Supplemental Study)</i>	M-11-00611	

† Four-Week Oral Gavage Toxicity Study Of TAK-375 In Rats	M-11-00027	
† Four-Week Oral Gavage Toxicity Study Of TAK-375 In Rats With A 13-Week Recover Period	M-11-00110	
Twenty-Six-Week Oral Gavage Toxicity Study Of TAK-375 In Rats	M-11-00176	
Preliminary Two-Week Oral Gavage Toxicity Study Of TAK-375 In Monkeys	M-11-00606	
Preliminary Two-Week Oral Gavage Toxicity Study Of TAK-375 In Monkeys (Supplemental Study)	M-11-00607	
† Four-Week Oral Gavage Toxicity Study Of TAK-375 In Monkeys	M-11-00026.001	
A 39-Week Oral Gavage Toxicity Study Of TAK-375 In Monkeys	M-11-00232	
† Preliminary One-Week Intravenous Toxicity Study Of Metabolite Of TAK-375, M-II(2S, 8S)(T-163302), In Monkeys	M-11-00156	
† Four Week Intravenous Toxicity Study Of TAK-375 In Monkeys	M-11-00192.001	
† Reverse Mutation Assay of TAK-375	M-11-00013.001	
† Concentrations Of Metabolites M-I, M-II, M-III, And M-IV Of TAK-375 In The Reaction Mixture For The Bacterial Reversion Assay After Incubating TAK-375 With S9 Mix.	M-11-00189	
† In Vitro Mammalian Cell Gene Mutation Test	M-11-00019.001	
† Concentrations Of Metabolites M-I, M-II, M-III, And M-IV Of TAK-375 In The Reaction Mixture For The Mouse Lymphoma Assay After Incubating TAK-375 With S9 Mix.	M-11-00186	
† Chromosomal Aberration Test On CHL Cells Treated With TAK-375	M-11-00017.001	
† Concentrations Of Metabolites M-I, M-II, M-III, And M-IV Of TAK-375 In The In Vitro Cytogenetic Assay After Incubating TAK-375 With S9 Mix.	M-11-00185	
† Micronucleus Test On TAK-375 In Mice	M-11-00020	
† Micronucleus Test In Rats Treated With TAK-375	M-11-00018	
† In Vivo/In Vitro Unscheduled DNA Synthesis (UDS) Assay With (UDS) TAK-375 In Rat Hepatocytes	M-111-00022	
Four-Week Oral Gavage Range-Finding Toxicity Study of TAK-375 in Mice	M-11-00609	4.2.3.3 Genotoxicity
Thirteen-Week Oral Gavage Range-Finding Toxicity Study Of TAK-375 In Mice	M-11-00178	
Twenty-Four-Month Oral Gavage Carcinogenicity Study Of TAK-375 In Mice	M-11-00560.002A	4.2.3.4 Carcinogenicity (4.2.3.4.3 Other Studies)

Four-Week Oral Gavage Toxicity Study Of TAK-375 In Rats With 13-Week Recovery Period	M-11-00608	
Thirteen-Week Oral Gavage Range-Finding Toxicity Study Of TAK-375 In Rats	M-11-00171.001	
Twenty-Four-Month Oral Gavage Carcinogenicity Study Of TAK-375 In Rats	M-11-00561.001A	
The Mutagenic, DNA-Reactive And Carcinogenic Potentials Of TAK-375 And Its Metabolites	M-11-00398	
Effects Of TAK-375 On Melatonin And Thyroid Hormone Levels And On Histology Of The Harderian Gland In Aged Mice	M-11-00709	
Effects Of Orally Administered TAK-375 On Plasma Hormone Levels In Rats	M-11-00073	
Effects Of TAK-375 On Plasma Estradiol And Prolactin Levels In Female Rats	M-11-00565	
Effects Of TAK-375 On Plasma Melatonin And Dehydroepiandrosterone (DHEA) Levels And On Histology Of Hepatocytes In Male Rats	M-11-00599	
Effects Of 7-Day Treatment With TAK-375 On Plasma Testosterone And Luteinizing Hormone Levels In Male Rats	M-11-00689	
Effects Of 7-Day Treatment With TAK-375 On Plasma Testosterone And Luteinizing Hormone Levels In Male Rats -- Supplemental Study	M-11-00690	
Effects Of 4-Week Treatment With TAK-375 On Plasma Testosterone And Luteinizing Hormone Levels In Male Rats	M-11-00691	

Studies not reviewed within this submission:

Validation Of An <u> </u> Method For The Determination Of TAK-375 And Its Metabolites, M-I, M-II, M-III And M-IV, In Mouse Plasma	M-11-00628	4.2.2.1/Analytical Methods & Validation Reports
Validation Of An <u> </u> Method For The Determination Of TAK-375 Metabolites, T-163302 And T-163306 (Diastereoisomers Of M-II), In Mouse Plasma	M-11-00629	
Validation Of A Method For The Determination Of TAK-375 In Rat And Monkey Plasma By <u> </u>	M-11-00630	
Validation Of An HPLC Method For The Determination Of TAK-375-375 In Rat	M-11-00631	

Plasma		
Validation Of An HPLC Method For The Determination Of TAK-375-375 In Rat Plasma	M-11-00632	
Validation Of An — Method For The Determination Of TAK-375 And Its Metabolites, M-I, M-II, M-III And M-IV, In Rat Plasma	M-11-00633	
Validation Of An — Method For The Determination Of TAK-375 And Diastereoisomers Of Metabolite M-II, T-163302 And T-163306, In Rat And Monkey Plasma	M-11-00634	
Validation Of An — Method For The Determination Of TAK-375 And Diastereoisomers Of Metabolite M-II, T-163302 And T-163306, In Rabbit Plasma	M-11-00635	
Validation Of An — Method For The Determination Of TAK-375 And Its Metabolites, M-I, M-II, M-III And M-IV, In Rabbit Plasma	M-11-00636	
Validation Of An — Method For The Determination Of TAK-375 Metabolites, M-I, M-II, M-III And M-IV, In The Reaction Mixture For The Ames Test	M-11-00637	
Validation Of An — Method For The Determination Of TAK-375 Metabolites, M-I, M-II, M-III And M-IV, In The Reaction Mixture For The In Vitro Cytogenetic Test	M-11-00638	
Validation Of An — Method For The Determination Of TAK-375 Metabolites, M-I, M-II, M-III And M-IV, In The Reaction Mixture For The Mouse Lymphoma Assay	M-11-00639	
Validation Of A Method For The Determination Of TAK-375 And Its Metabolite M-II In Rat Plasma And Brain Homogenate Using []	M-11- 776	
Validation Of A Method For The Determination Of TAK-375 And Its Metabolite M-II In Monkey Plasma Using []	M-11-777	
Preliminary Two-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys	M-11-00606	4.2.3.2 Repeat-dose Toxicity
Preliminary Two-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys (Supplemental Study)	M-11-00607	
Preliminary One-Week Intravenous Toxicity Study of Metabolite of TAK-375, M-II(2S, 8S)(T-163302), in Monkeys	M-11-00156	
Range-Finding Study for Effects of	M-11-00168	4.2.3.5 Reproductive and

Developmental Toxicity		
Intravenously Injected TAK-375 M-II (2S, 8S), a Metabolite of TAK-375, on Embryofetal Development in Rats		4.2.3.7 Other Toxicity Studies (impurities)
Oral Single Dose Toxicity Study of TAK-375 Containing New Related Substance [] in Rats	M-11-00098	
Four-Week Oral Gavage Toxicity Study of TAK-375 (Lot No. M375-017) in Rats	M-11-00356.001	
Bacterial Reversion Assay with TAK-375 (Lot No. M375-010)	M-11-00052	
Bacterial Reversion Assay with TAK-375 (Lot No. M375-017)	M-11-00353	
Cytogenetic Assay with TAK-375 (Lot No. M375-017) in Chinese Hamster Lung (CHL) Cells	M-11-00354	

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Melatonin is an endogenous hormone found in both the vertebrate retina and pineal gland. Melatonin is secreted by the pineal principally at night and functions as an autocrine, paracrine and endocrine signal depending on the location receptors are found. The release of melatonin is primarily under the control of the suprachiasmatic nucleus of the hypothalamus (SCN) which is the output nucleus involved in the generation of the circadian rhythm and is itself influenced by melatonin and environmental light. Melatonin has been proposed as a "chronobiotic," defined as a treatment which can alter the body's circadian rhythm. The role of melatonin in sleep is not well understood but is hypothesized that nighttime release and stimulation of the SCN is involved in stabilizing the circadian rhythm output of the nucleus. Shift-work, time-shifts due to travel across time zones are thought to weaken the circadian rhythm output of the SCN and lead to a decrease in melatonin release and disruption of sleep. The natural reduction of melatonin secretion as a function of aging is also hypothesized to underlie a weakening of the SCN circadian rhythm and consequent impairment of sleep resulting in primary insomnia commonly found in the elderly.

Melatonin receptors are found distributed throughout nearly all tissues of the body. Animal studies investigating 2-[¹²⁵I]-iodomelatonin-binding revealed significant binding of this specific melatonin receptor agonist in the pars tuberalis of the adenohypophysis, superior colliculus and neural retina (thought involved in visual function), SCN, arteries, and reproductive tissues including the epididymis in the male, granulosa of preovulatory follicles, and prostate epithelium. Since 1998, melatonin receptors have been classified by molecular and pharmacological analysis into 3 subtypes: high-affinity *mt1* and *MT2* receptors ($K_d = 30-150$ pM) which were formerly classified as subtypes of the *ML1* receptor (*ML_{1A}* or *ML_{1B}*, respectively; also *mel_{1a}* and *mel_{1b}*, respectively), and lower-affinity *MT3* receptors which exhibit nanomolar affinity for melatonin [see figure from Dubocovich et al., 1999, below].

	mt ₁	mt ₂	MT ₃
Nomenclature			
Other Names	Me ₁ , ML _{1A}	Me ₂ , ML _{1B}	ML ₂
Affinity (K _D)	45 pM	140 pM	0.3-2 nM
Pharmacology	MLT >> NAS	MLT >> NAS	MLT = NAS
Selective Agonists	---	---	N-Acetyl 5HT 5-MCA-NAT
Selective Antagonists	---	4P-ADOT 4P-PDOT	PRAZOSIN

Figure 1. Nomenclature for mammalian melatonin receptors. The nomenclature described here was adopted by the Nomenclature Committee of International Union of Pharmacology in 1998. For further details please see reference 25. Luzindole: MLE; melatonin (N-acetyl-5-methoxytryptamine); NA 5-HT; N-acetyl-5-hydroxytryptamine; 5-MCA-NAT: 5-methoxy-carboxylamino- N-acetyltryptamine; 4-P-PDOT: 4-phenyl 2-propionamidotetraflinone; 4-P-ADOT: 4-phenyl 2-acetamidotetraflinone.

The mt1 and MT2 receptors have been cloned and demonstrate 60% homology between each other and are members of the G-protein-linked 7-transmembrane superfamily. Functionally these receptors have been shown to inhibit cAMP production through pertussis-sensitive Gi protein inhibition of adenylate cyclase. A parallel stimulation of PGF2 α leading to PLC β activation has also been demonstrated resulting from release of the $\beta\gamma$ -subunits of the G γ -protein. The MT3 receptor is believed to be a melatonin-sensitive quinone reductase 2 enzyme and will not be discussed further as TAK-375 was not studied for interaction with this melatonin-sensitive enzyme.

The affinity and potency of TAK-375 for melatonin receptor subtypes as well as other receptors were tested in a series of *in vitro* binding and functional studies. TAK-375 and an active metabolite, M-II were also tested in *in vivo* studies in animals to assess the potential efficacy of TAK-375 and an active metabolite, M-II, for sleep promotion in these models.

TAK-375 and its active metabolite M-II appear to be high affinity, high potency full agonists the ML₁ receptors. Binding studies in cell lines transfected with melatonin receptor subtypes as well as in tissue homogenates expressing ML₁ (i.e. MT1 and MT2 receptors) or ML2 (i.e. MT3) receptors indicated TAK-375 has picomolar affinity for MT1 and MT2 receptors. TAK-375 binding affinity (K_d) for MT1, MT2 and MT3 receptors was estimated at 11.6 – 14 pM, 112 pM, and 2.65 μ M, respectively. TAK-375 therefore shows a 25,000 - 190,000-fold binding differential between the ML₁ receptor subtypes and the ML2 (MT3) receptor subtype. The M-II metabolite of TAK-375 exhibits a K_d ~ 114 pM at the MT1 receptor and 566 pM at the MT2 receptor. TAK-375 therefore demonstrates a 5-10-fold higher affinity for the ML₁ receptor subtypes when compared with its metabolite M-II. Functional studies using TAK-375 and M-II revealed picomolar inhibition of forskolin-stimulated cAMP accumulation with the TAK-375 having approximately 17 – 25-fold higher potency than the metabolite M-II. Both TAK-375 and M-II demonstrated little affinity or efficacy at non-ML₁ receptor sites when tested in a broad screen of receptors and enzymes though M-II did demonstrate inhibition of 5-HT2B receptor function at with a K_i ~ 1.7 μ M, representing 1000-fold lower potency than M-II at the MT1 receptor.

Studies in instrumented cats and rhesus monkeys revealed oral administration of TAK-375 to produce decreased wakefulness, increased slow wave sleep and an increase in rapid eye movement (REM) sleep at a dose of 0.1 mg/kg in cats while administration of TAK-375 at doses ≥ 0.03 mg/kg in rhesus monkeys produced a decreased latency to light sleep and a decreased latency to slow wave sleep as well as a total increase in sleep duration. Sleep architecture was not observed to be altered nor was myorelaxation noted in contrast to zolpidem used as an active control.

Other findings:

- TAK-375 at doses ≥ 0.1 mg/kg p.o. promoted reentrainment of circadian wheel running in rats subjected to an 8-hr phase advancement in their light/dark cycle. This effect was similar to that found with melatonin at 10 mg/kg p.o.
- TAK-375 administered at doses up to 30 mg/kg p.o. to rats did not affect responses in the Morris Water Maze and the Delayed Matching to Position tasks suggesting there was little effect of TAK-375 on learning and memory under the conditions tested.
- TAK-375 administered at doses of 0.3 or 3 mg/kg p.o. to rats for 14-days at 6 p.m. did not affect nocturnal plasma concentrations of melatonin as measured at 8 pm, midnight, 4 a.m. and 8 a.m. This reviewer noted a non-significant reduction of approximately 38% in plasma melatonin at the 4 a.m. timepoint, however. Note that toxicology studies conducted in rat demonstrated that at higher doses (≥ 60 mg/kg/day) TAK-375 administration was associated with increased plasma melatonin levels in rats.
- TAK-375 at doses ≥ 50 mg/kg p.o. in monkeys are associated with a dose-dependent increase in emesis, episodes of which could occur up to 6 hr after drug administration. Blockade of emesis could be achieved with either luzindole, an ML_1 receptor antagonist, or granisetron, a 5-HT₃ receptor antagonist indicating this response is an extension of the pharmacologic action of TAK-375 with 5-HT₃ receptors at a critical juncture in the pathway mediating this effect. TAK-375 appeared to be slightly more potent at causing emesis than melatonin, in keeping with their pharmacologic profile at ML_1 receptors.
- CNS safety pharmacology studies indicated general effects consistent with TAK-375 being a mild CNS depressant or hypnotic at doses up to 100 mg/kg p.o. in mice. TAK-375 reduced the severity of seizures in mice and increased pentobarbital sleeping time in rats. TAK-375 administered at a dose of 100 mg/kg p.o. to rats had no effect on spontaneous locomotion or a measure of pain response. Body temperature was unaffected at 100 mg/kg p.o. in rats as well.
- TAK-375 produced no alterations in ECG waveforms with administration of 66 mg/kg p.o. to dogs. Adequate exposure to TAK-375 was demonstrated in this model though the M-II metabolite was not assessed therefore adequate exposure to this metabolite could not be confirmed. A sheep purkinje fiber study demonstrated no effect of TAK-375 on the shape of the action potential below 10 μ M but at 100 μ M there was a shortening of the action potential duration. The M-II metabolite was not tested in this *in vitro* model, however. The M-II metabolite as well as TAK-375 were demonstrated to lack effects on the delayed rapid outwardly-rectifying potassium channel current, I_{Kr} , in a hERG channel assay at concentrations up to 100 μ M. An *in vivo* study in instrumented cynomolgus monkeys with TAK-375 administration up to

200 mg/kg demonstrated slight reductions in heart rate and an increase in mean blood pressure with the 200 mg/kg dose. Additionally, body temperature and spontaneous locomotor activity was observed to decrease. Repeated administration of 200 mg/kg/day for 5 days of repeated dosing demonstrating no tolerance to these effects developed at this dose. M-II would not have been adequately evaluated in this study as this metabolite does not circulate in the plasma of cynomolgus monkeys in significant concentrations.

- Pulmonary effects were not observed with TAK-375 administration at a dose of 66 mg/kg in the conscious dog study described above.
- No effects of TAK-375 on urine volume or urine electrolytes were observed with doses up to 100 mg/kg in rats.
- Mild inhibitory effects of TAK-375 on GI motility and gastric emptying were noted in rats administered TAK-375 100 mg/kg p.o.
- TAK-375 was assessed for the ability to produce physical dependence and withdrawal in rats and cynomolgus monkeys. No evidence for physical dependence or withdrawal was found. Rats administered TAK-375 up to 600 mg/kg/day for 4 weeks followed by cessation of dosing did not demonstrate evidence of withdrawal. Monkeys administered TAK-375 10 mg/kg/day i.v. for 52 weeks did not demonstrate evidence of withdrawal during 5-day discontinuation periods during Weeks 14, 27, 40 and 53. Monkeys trained to discriminate benzodiazepine-like subjective effects from vehicle responded to TAK-375 10 mg/kg i.v. at rates comparable to vehicle. Reinforcing effects of TAK-375 were evaluated in rats and monkeys. Rats administered up to 100 mg/kg TAK-375 p.o. did not demonstrate place-preference unlike positive controls. In monkeys, TAK-375 administered up to 2 mg/mL i.v. produced responding which was more similar to vehicle than the positive control, methohexital in 3/4 monkeys, though one monkey exhibited a slight increase in self-administration behavior with TAK-375. No explanation for the different response in this monkey was provided.
- TAK-375 administered at doses up to 30 mg/kg in rats demonstrated no evidence of pharmacodynamic interaction with the benzodiazepine diazepam on rota-rod performance.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

TAK-375 was tested in *in vitro* binding studies for its affinity to various melatonin receptors including the human $mt1$ and $MT2$ (ML_1) receptors stably transfected into CHO cells, chick ML_1 and hamster MT_3 (ML_2) receptors from brain homogenates [M-11-00617]. TAK-375 was demonstrated to bind with high affinity to the human mt_1 receptor ($K_d = 14$ pM), 6-fold higher than melatonin. Similarly, high affinity binding to chick brain homogenate expressing ML_1 receptors revealed a single-site with a $K_d = 23.1$ which represents a 16-fold greater affinity than that of melatonin. In contrast, hamster brain homogenates expressing the MT_3 (ML_2) binding site showed only low affinity binding with TAK-375 with a $K_d = 2.65$ μ M, an affinity approximately $1/110^{th}$ that of melatonin. The apparent relative affinity of TAK-375 for ML_1 receptors was nearly 115,000 times greater than for the ML_2 receptor as expressed

in the hamster brain. TAK-375 additionally was quite potent at inhibiting forskolin-induced cAMP production with an IC_{50} of 21.2 pM. Further study [M-11-00616001r] of TAK-375 as well as the active metabolite M-II (2S, 8S) in an *in vitro* binding assay with CHO cells stably transfected with the human MT_2 (Mel_{1b}) receptor indicated a similarly high affinity of TAK-375 for this subtype of the ML_1 receptor with a $K_d = 112$ pM. M-II also demonstrated a strong affinity for the MT_2 receptor ($K_d = 566$ pM) though this represented approximately 5-fold lower affinity for this receptor as compared to the parent. Both TAK-375 and M-II were shown to be potent and full agonists *in vitro*, with inhibition of forskolin-stimulated cAMP production associated with IC_{50} values of 53.4 pM (TAK-375) and 1,470 pM (M-II) in CHO cells transfected with the human MT_1 receptor gene. At maximal concentrations used, both TAK-375 and M-II were able to reduce forskolin-stimulated cAMP production by more than 80%. Although TAK-375 is nearly 28-fold more potent than M-II in this assay it is observed that melatonin was only slightly more potent than M-II ($IC_{50} = 980$ pM [melatonin] vs. 1,470 pM [M-II]). Taken together, TAK-375 and M-II appear to have high affinity for and potency at ML_1 receptors and function as full agonists at these sites and, for at least TAK-375, is unlikely to have activity at the MT_3 (ML_2) binding site due to its low affinity for the receptor. *M-II has not been assessed at this site, however.* Exploration of the remaining TAK-375 metabolites for various melatonin receptors was conducted in a study using CHO cells stably transfected with the Mel_{1a} gene (CHO-hMel R7 — which express the human MT_1 receptor, chick forebrain homogenate containing ML_1 receptor and Syrian hamster brain homogenate containing the ML_2 receptor. TAK-375 and its metabolites M-I through M-V demonstrated little to no affinity for the ML_2 receptor ($K_i > 3,500$ nM). In agreement with a previously described study M-II (2S, 8S) demonstrated strong affinity for the MT_1 receptor expressed in CHO cells ($K_i \approx 114$ pM) which was approximately 1/10 the affinity observed for TAK-375. The affinity of M-II for the ML_1 receptor as determined in binding assays conducted using chick brain indicated this metabolite has an affinity that is 30-fold lower than TAK-375 though this still represents a very high affinity interaction (see Sponsor's table below). Though M-V appeared to have high affinity for the ML_1 receptor in chick brain homogenates as well ($K_i \approx 4.0$ nM) this was approximately 200-fold weaker than that observed with TAK-375. A relative comparison of TAK-375 with metabolites for affinity to ML_1 receptors of the chick brain is reproduced from Sponsor's submission below.

Affinities of TAK-375 and Metabolites for the ML_1 Receptor in Chick Brain

Compounds	K_i (pmol/L)	Relative affinities
TAK-375	19.9 ± 1.23	1
M-I	>236000	<0.000085
M-III	36000 ± 1230	0.00055
M-IV (2S, 8R)	>236000	<0.000085
M-V	4040 ± 97.5	0.0049
TAK-375	21.4 ± 2.23	1
M-II (2S, 8S)	654 ± 74.3	0.033

Each K_i value shows the mean ± S.E.M. of 3 experiments done in duplicate and was expressed with three significant digits.

Relative affinities are expressed with two significant digits.

Also in general agreement with a previous study, the M-II metabolite was shown to be active as an agonist at mt_1 receptors, decreasing forskolin-induced cAMP accumulation with a

potency that was approximately 17-fold lower than TAK-375 and 4-fold lower than melatonin (see Sponsor's table below).

Affinity and Potency of M-II (2S, 8S) Compared to TAK-375 and Melatonin at the Cloned Human MT₁ Receptor

Compounds	Receptor binding K _i (pmol/L)	cAMP production IC ₅₀ (pmol/L)
M-II (2S, 8S)	114 ± 12.5	208 (60.4 - 850)
Melatonin	45.4 ± 4.11	48.1 (36.9 - 63.0)
TAK-375	11.6 ± 0.866	12.4 (10.9 - 14.3)

Each K_i value shows the mean ± S.E.M. of 3 (TAK-375 and M-II (2S, 8S)) and 6 (melatonin) experiments done in duplicate.

Each IC₅₀ value was calculated from 2 experiments done in triplicate.

Figures in the parentheses indicate 95% confidence intervals of IC₅₀ values.

Values are expressed with three significant digits.

An *in vitro* study of neonatal rat pituitary response to TAK-375 in a model of forskolin-stimulated cAMP production [M-11-00619] provided evidence that at doses of 0.03 – 0.3 nM of TAK-375 reduced cAMP levels up to 67.5% while melatonin used at the identical concentrations was observed to produce an even greater inhibition of 96%.

Drug activity related to proposed indication:

TAK-375 was administered by oral route as a solution of test article in 0.5% methylcellulose in capsule to instrumented, freely-moving cats and rhesus macaque monkeys to characterize the potential hypnotic effects of this compound and more specifically on sleep parameters and architecture.

TAK-375 (0.0001, 0.001, 0.01 and 0.1 mg/kg), melatonin (0.001, 0.01, 0.1 or 1.0 mg/kg) or vehicle (0.5% w/v methylcellulose) was administered to adult male and female cats orally in a crossover design study with 7 day washout between test article trials [study M-11-00613]. Cats previously underwent stereotaxic implantation of electrodes to monitor electroencephalographic (EEG) activity with bilateral electrodes placed in the frontal and parietal regions of the skull and a deep electrode implanted into the hippocampus to measure theta waves. Subcutaneous electrodes were implanted into the back cervical muscles to obtain the electromyogram (EMG) and screws were placed above the bony orbit of the eye to obtain the electro-oculogram (EOG) which was used to assess REM activity. Animals were allowed to recover and were habituated to the test environment. Recording sessions were 9 hr. in length with the first hour being prior to drug treatment and 8 hr. post-administration. Sleep status was classified into one of three stages: 1) wakefulness, 2) slow wave sleep (SWS), 3) Rapid Eye Movement (REM) sleep. TAK-375 administration produced dose-dependent effects which appeared to indicate the promotion of sleep. At the high dose of 0.1 mg/kg p.o. TAK-375 was associated with ↓% wakefulness, ↑%SWS and an ↑%REM sleep for up to 6 hr after drug administration (at the 2, 4 and 6 hr time-points). TAK-375 at 0.001 and 0.01 mg/kg p.o. produced similar effects on wakefulness and slow wave sleep but did not increase REM sleep as did the high dose. Also, while 0.01 mg/kg TAK-375 appeared to

exert an effect throughout 6 hr post-administration, 0.001 mg/kg TAK-375 was only different from control at the 6 hr time-point and was not different at either 2 or 4 hr after drug administration.

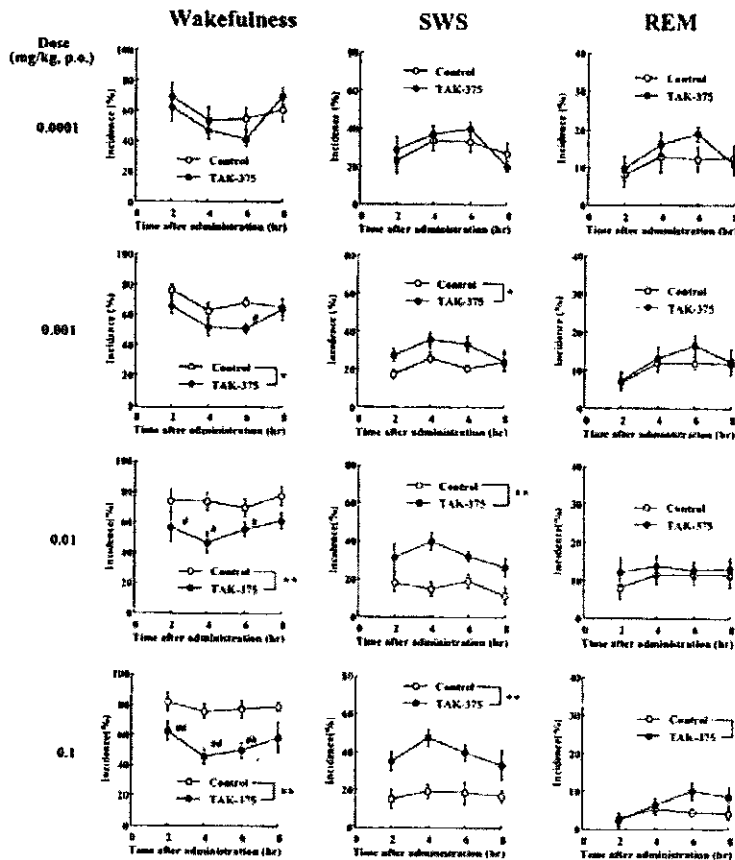


Figure 1 Effects of TAK-375 on sleep and wakefulness in freely moving cats. Each value shows the mean percentage of wakefulness, SWS, or REM stage during each block of 2 hours with S.E.M. Eight cats were used in each group. * $p \leq 0.05$, ** $p \leq 0.01$, compared with the vehicle-treated control (ANOVA). # $p \leq 0.05$, ## $p \leq 0.01$, compared with the vehicle-treated control (paired t test with Holm correction).

Sleep latency (time to first SWS episode) tended to be reduced but this did not reach statistical significance (not shown). Melatonin produced similar effects as TAK-375 but was generally weaker and effects were only observed at the 2 hr time-point (not shown).

A subsequent study [M-11-00624.001R] was conducted in cats and using oral administration of the major human metabolite M-II (2S, 8S) at doses of 0.001, 0.01, 0.1 and 1.0 mg/kg for the purpose of determining whether the apparent *in vitro* affinity and potency of the metabolite compared with the parent TAK-375 for the ML_1 receptor would be replicated *in vivo*. Cats were instrumented as described in the previous study and M-II dose levels were compared against vehicle in a crossover design. No effect was observed with M-II administration at the lowest dose, 0.001 mg/kg, but higher doses of M-II produced dose-dependent effects on sleep. The high and high-mid doses of 1.0 and 0.1 mg/kg produced a significant $\downarrow$ %wakefulness (incidence) and a $\uparrow$ %SWS (incidence) for 6 hr while the 0.01

mg/kg dose level was associated with a ↑%SWS and a trend towards ↓%wakefulness that did not reach statistical significance at this dose. No effects on REM sleep were noted at any M-II dose level. Thus, the effects of the M-II metabolite on sleep augmentation were qualitatively similar to that of the TAK-375 parent though the metabolite appeared to be approximately 10-100 – fold less potent than TAK-375; a finding which correlates well with the *in vitro* affinity and potency comparison between the parent and metabolite.

A polysomnographic (PSG) study [M-11-00572] was conducted in female rhesus monkeys in a manner similar to the aforementioned studies in the cat to determine if the hypnotic effects observed in cats could be replicated in a non-human primate. TAK-375 (0.003, 0.03 and 0.3 mg/kg), melatonin (0.3, 1 and 3 mg/kg) and zolpidem (1, 3, 10 and 30 mg/kg) were administered to monkeys instrumented for recording of EEG, EMG and EOG. Each animal underwent two vehicle (0.5% methylcellulose) trials, once before and a second trial after drug trials were conducted with the control values being the average of the two vehicle trials. TAK-375 at the lowest dose was not observed to produce an effect on any measured parameter when compared with control trials but the mid and high dose of 0.03 and 0.3 mg/kg produced a decreased latency to light sleep (LS) and slow wave sleep (SWS) as well as a total increase in sleep duration (see Sponsor's figures below).

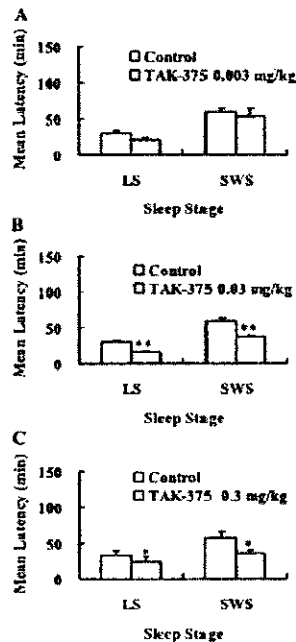
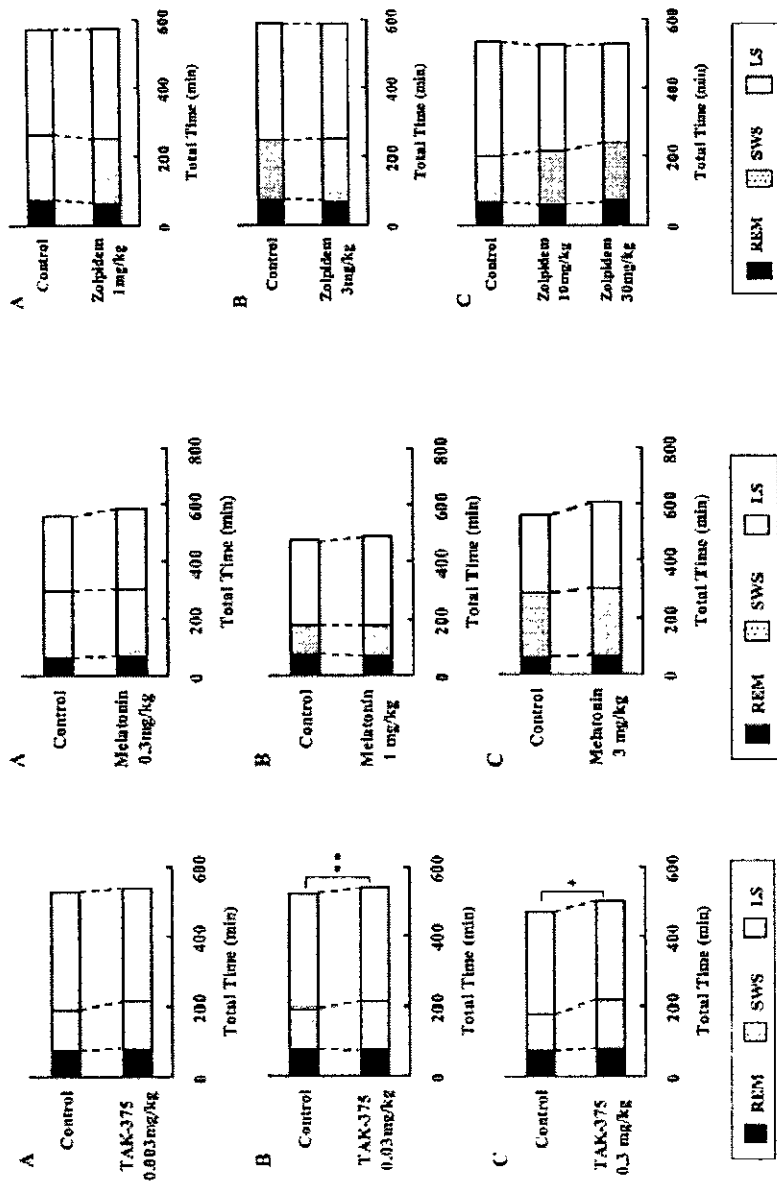


Fig. 1. Effects of TAK-375 on latency to sleep onset. Each value shows the mean latency (min) ± SE to each sleep stage at the dose of 0.003 (A, n=5), 0.03 (B, n=6) or 0.3 mg/kg (C, n=6). *p<0.05, **p<0.01 compared with the control treated vehicle (paired t-test with Holm's correction).



Effects of TAK-375, melatonin and zolpidem administered to rhesus monkeys on the amount of each sleep stage at nighttime. Each value shows the mean duration (min) ± SE of each stage at the doses listed (n = 4-6 animals/group). *p<0.05, **p<0.01, compared with the control treated vehicle (paired t-test).

An analysis of sleep stage duration suggested a trend towards the lengthening of the SWS stage though this did not reach statistical significance. At the mid and high dose level of TAK-375, the sleep alterations observed did not result in a change in sleep architecture. This is in contrast to findings observed with high dose zolpidem which increased high frequency EEG activity compared to control sleep as well as inducing myorelaxation at the top dose of 30 mg/kg p.o. Zolpidem had no effect on latency to light sleep or total sleep time but exhibited a non-significant trend towards increasing SWS duration. Melatonin administered up to 3 mg/kg produced an increased total sleep time in monkeys but was not associated with statistically significant changes in the characteristics of any interior sleep stage. Therefore these changes appeared to be subtle and only observed upon a global assessment of sleep pattern in the monkey. As with TAK-375 (and unlike zolpidem) no alterations in sleep architecture were found to occur along with the increased TST parameter. The active metabolite of TAK-375, M-II (2S, 8S) was also studied for sleep promotion in rhesus monkeys with dosing of 1, 3 and 10 mg/kg p.o. which was compared with vehicle trials [M-11-00736]. Results indicated that in agreement with the findings of M-II in the cat, the active metabolite produced similar effects as the parent TAK-375 though with reduced potency. M-II produced a decreased latency to LS and SWS and did not affect the architecture of sleep in this species at the 3 mg/kg dose level. Decreased latency to sleep stages was not observed with either the low dose or high dose, however. Total Sleep Time evaluation indicated a trend toward increased values but this did not reach statistical significance, however (see Sponsor's figures below).

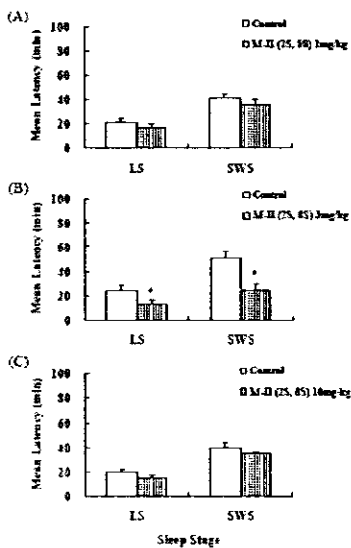


Figure 1. Effect of M-II (2S, 8S) on latency to sleep onset. Each value shows the mean latency (min) ± SE to each sleep stage at the dose of 3 (B) or 10 mg/kg (C) (n=5). *p<0.05 compared with the control treated vehicle (t-test with Holm correction)

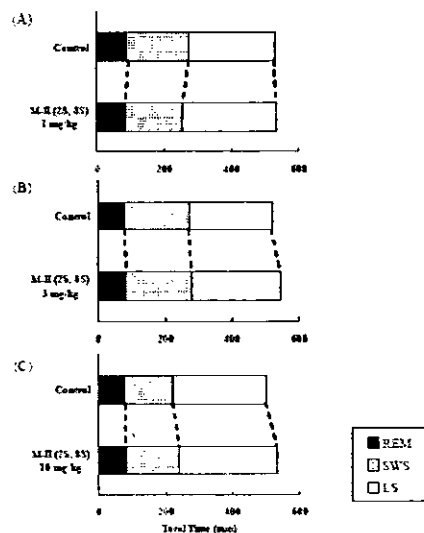


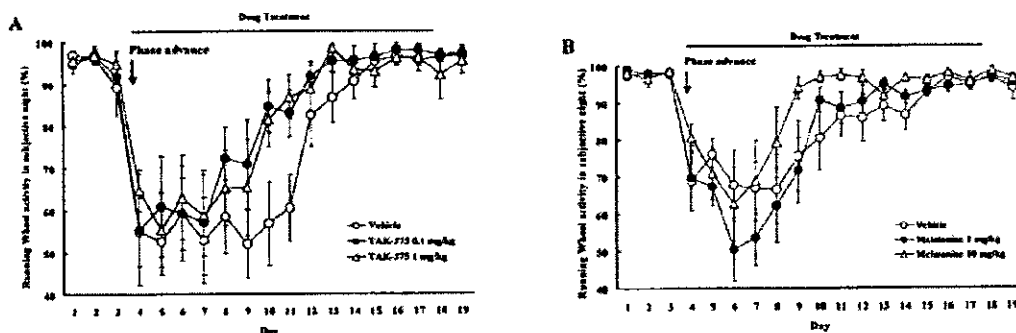
Figure 2. Effect of M-II (2S, 8S) on the amount of each sleep stage at nighttime (12hrs: 18.00-6.00). Each value shows the mean duration (min) of each stage at the dose of 1 (A) 3 (B) or 10 mg/kg (C) (n=5).

2.6.2.3 Secondary pharmacodynamics

TAK-375 and M-II receptor pharmacology (alternative sites): TAK-375, the active metabolite M-II and melatonin were tested in a broad screen of receptor and enzyme affinity using competitive binding assays as well as functional inhibition assays [

] [studies M-11-00618 and M-11-00389]. Incubation of receptor targets with 10 μ M TAK-375, the active metabolite M-II or melatonin demonstrated little to no “off-target” affinity at 129 potential receptor and transporter binding sites. TAK-375 did not show > 50% inhibition of competitor binding any of the receptors in the assay screen. Importantly, TAK-375 did not exhibit significant affinity for opioid receptors, GABA_A receptors, benzodiazepine binding sites, or dopamine reuptake transporters at 10 μ M. M-II, however, demonstrated a weak affinity for 5-HT_{2B} receptors ($K_i \sim 1.75 \pm 0.2 \mu$ M) but did not inhibit the binding of specific ligands at any other site with > 50% effectiveness. Melatonin demonstrated only weak affinity for the 5-HT_{1A} receptor with a $K_i \sim 5.6 \mu$ M. Incubation of various enzymes (approximately 75) with their specific substrates and TAK-375 and M-II at concentrations of 10 – 1000 μ M demonstrated no appreciable interference at tested concentrations. Melatonin tested at identical concentrations also failed to interfere with substrate specific interactions with these enzymes of interest.

Circadian rhythm re-entrainment: TAK-375 effects on circadian rhythm re-entrainment has been studied by the Sponsor using the rat wheel running model, a behavior which demonstrates strong dependence on circadian rhythm and is almost entirely observed at (subjective) night [M-11-00323.001R]. Rats were allowed to wheel run for 21 days under a 12hr lights on/lights off (lights on at 7 a.m.) condition for 21 days prior to the light/dark cycle being phase advanced 8 hrs (i.e. lights on at 11 p.m.). On the day of phase advancement, TAK-375 (0.1 or 1 mg/kg p.o.), Melatonin (1 or 10 mg/kg p.o.) or vehicle (0.5% methylcellulose) was given immediately prior to lights on 11 p.m. at the start of the phase-advance schedule and was continued for 14 days during while the alternate light/dark schedule was in effect. TAK-375 administration at doses of 0.1 and 1.0 mg/kg p.o. were demonstrated to promote the advancement of circadian rhythm activity by shortening the latency to appropriate wheel running behavior during the newly defined night. On the 7th and 8th day after phase advance, the re-entrainment percentages for vehicle-treated mice were 56.7 and 60.4%, respectively while in TAK-375-treated rats given 0.1 mg/kg p.o. the re-entrainment percentages were 84.6 and 82.9% on the 7th and 8th day while at the 1.0 mg/kg dose re-entrainment was 81.4 and 86.9%. Melatonin had a similar effect at 10 mg/kg but was without effect at 1 mg/kg p.o. Vehicle-treated rats reached 90% re-entrainment after 11 days in the phase-advance setting while TAK-375 animals reached this level in 9 days (0.1 mg/kg) and 10 days (1.0 mg/kg). The results of this study indicate that TAK-375 appears to accelerate the re-entrainment of circadian rhythm behavior when challenged by light/dark cycle phase-advance.



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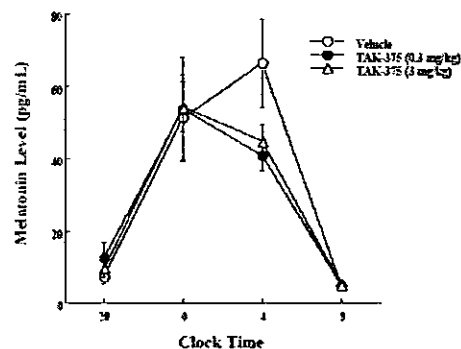
Learning & Memory: To determine if TAK-375 is likely to exert an effect on learning and memory, the Sponsor employed two animal models commonly used for this purpose: the Morris Water Maze task and the Delayed Matching to Position (DMTP) assessment [M-11-00620]. Positive controls used in these studies were two common benzodiazepines, diazepam and triazolam, which are well known to cause deficits in these neurologic parameters. TAK-375 was given as 10, 30 or 100 mg/kg by oral gavage 30 minutes prior to placement in the Morris Water Maze on 3 subsequent trial days. Each trial required the rat to find a platform submerged in the same location in a water tank and over the three trials the vehicle control animals demonstrated quick improvement in the time necessary to find the platform, indicative of acquisition of a learned task. TAK-375 up to a dose of 30 mg/kg p.o. did not affect the acquisition of this task. Melatonin up to 100 mg/kg p.o. similarly failed to affect task acquisition. According to the Sponsor's interpretation, diazepam and triazolam showed a dose-dependent impairment of task acquisition though this reviewer's assessment of these trials indicates the positive controls used in the assay did not demonstrate test sensitivity. On the first trial there was already an obvious difference between benzodiazepine-treated animals and vehicle-treated animals on the time necessary to find the platform and this difference decreased markedly over the 3 trials indicating appropriate acquisition of this task. This could indicate the animals had decreased anxiety, reduced motor activity or were slightly uncoordinated at the initial trial and habituated to the presence of drug over the successive two trials. The ideal positive control would have produced a response that was indistinguishable from vehicle animals on Trial 1 but showed little to no reduction in the time necessary to find the platform over the subsequent trials. Thus, the study lacks a valid positive control which would demonstrate assay sensitivity. Nevertheless, the study does not suggest an obvious impairment of neurologic function at the doses of TAK-375 administered to rats. In the second study in which memory in rats was assessed by means of the Delayed Matching To Position (DMTP) test, no effect of TAK-375 up to an oral dose of 30 mg/kg was observed on this measure nor did melatonin impair memory formation. Diazepam and triazolam, in contrast according to Sponsor, produced a significant decrease in the ability of rats to press the correctly associated lever when a delay between presentation of the light stimulus and the presentation of several levers. Similarly to the first experiment, this reviewer believes the positive controls did not establish test sensitivity as there was a reduction in the percentage of correct lever presses even when there was no delay between light stimulus and lever presentation. A true positive control would have not shown a reduction in correct responses when there was no delay but would increase

as the delay between light stimulus and lever presentation increased. Nevertheless, TAK-375 up to 30 mg/kg p.o. did not appear to present an obvious impairment of memory.

TAK-375 effect on plasma melatonin level: The Sponsor conducted a study [M-11-00623] designed to examine if TAK-375, as a melatonin receptor agonist, itself produces effects on nocturnal plasma melatonin levels. In a study of 6 rats/group, animals were administered either vehicle (0.5 w/v% MC), TAK-375 0.3 mg/kg or 3 mg/kg nightly at 5:30-6 pm (1-1.5 hr before lights off) for 14 days. On the final day of the study, blood was sampled at 8 pm, midnight, 4 am and 8 am and analyzed by means of a melatonin radioimmunoassay for plasma concentration.

Sponsor reported that no effect of TAK-375 on plasma melatonin levels at the midnight time-point compared with control and that an apparent melatonin reduction observed in the plasma of rats at both TAK-375 doses at 4 a.m. was not statistically significant (Sponsors figure, right).

The Sponsor suggests that TAK-375 may be advancing the phase of melatonin secretion but concludes that TAK-375 does not alter nocturnal plasma melatonin levels in rats. This reviewer notes that TAK-375 at doses of 0.3 mg/kg and 3.0 mg/kg was no different from vehicle at the midnight assessment but was associated with a 38% and 32% reduction in plasma melatonin levels, respectively, when compared with control values at 4 a.m. Although a dose-dependent effect of TAK-375 on plasma melatonin levels was not observed and the changes did not reach statistical significance, this study may still suggest a biologically significant effect of TAK-375 to reduce nocturnal plasma melatonin levels. Indeed, the reviewer's analysis of the confidence interval surrounding the difference between the means of the vehicle and high dose group suggests the true difference could be as high as 47 pg/mL (21 ± 26 pg/mL). The study as designed does not appear to be adequately powered to detect moderate differences between treatment groups.



The emetic potential of TAK-375 compared with melatonin in monkeys: Studies of TAK-375 in monkeys have revealed an emesis with doses above 50 mg/kg p.o., well above the apparent sleep-promoting effects at 0.3 mg/kg p.o.). The Sponsor wished to characterize the emetic potential of TAK-375 versus melatonin and furthermore determine if emesis was mediated through a direct effect on melatonin receptors or through other pathways such as the serotonergic pathway. Cynomolgus monkeys were administered TAK-375 200 mg/kg p.o. (in 0.5 w/v% MC) or melatonin given at either 200 or 1000 mg/kg (in 0.5 w/v% MC) and the number of vomiting episodes was counted over 6 hr. Administration of TAK-375 200 mg/kg and melatonin 1000 mg/kg produced significant vomiting (5.2 and 8.7 vomits/6 hr, respectively) in all animals tested with a moderately long latency (2.5 and 5.1 hr, respectively) while melatonin 200 mg/kg was less emetic (1.3 vomits/6 hr) and in fewer animals. Pretreatment with either lundazolidine (a ML-1 receptor antagonist) or granisetron (a 5-HT₃ receptor antagonist) 30 min prior to oral TAK-375 200 mg/kg administration produced a marked and quantitatively similar reduction in both the number of affected monkeys and number of vomiting episodes. Granisetron pretreatment also appeared to effectively reduce the number of melatonin-treated monkeys affected as well as the frequency of vomiting in though the latter did not reach statistical significance. Therefore, TAK-375 is slightly more

potent at producing emesis than melatonin. Furthermore, this emetic effect appears to be due to a direct effect on melatonin receptors due to the ability of selective ML-1 antagonists to attenuate the response and the apparent difference in potency of the two compounds paralleling their difference in receptor affinity *in vitro* and other pharmacodynamic effects *in vivo*. The response also appears to involve the serotonergic system through the demonstration that a 5-HT₃ receptor antagonist is as effective as the ML-1 receptor agonist at attenuating this response. The Sponsor believes that the large difference between efficacious doses for promoting sleep and those producing emesis combined with the demonstration of 5-HT₃ receptor involvement may argue this emetic response is a non-specific or off-target effect. In the opinion of the reviewer this does not appear to be necessarily true as TAK-375-associated emesis is likely an extension of ML-1 receptor agonist pharmacology (demonstrated also by the emetic effect of melatonin itself) which proceeds through a 5-HT₃ receptor-containing circuit.

2.6.2.4 Safety pharmacology

Neurological effects:

Effects of TAK-375 on behavior and CNS function were evaluated in comparison to vehicle and positive controls in a series of independent evaluations contained in a single report [M-11-00106.001A]. Oral administration of TAK-375 at a dose of 100 mg/kg to mice resulted in a reported light sedation when compared with animals administered vehicle. Behavioral effects noted included palpebral closure and decreased spontaneous locomotor activity with prone position notable within 30 minutes after dosing. All animals were considered normal by 60 minutes following drug exposure. Several test of CNS effects revealed effects which would be considered typical of a mild CNS depressant or hypnotic including prevention of tonic extensor seizures in mice and an increase in pentobarbital-induced sleeping time ($\uparrow 25 - 37\%$ vs. control) in rats. Cats given 10 mg/kg orally demonstrated decreased arousal by EEG analysis. No effects of TAK-375 were observed at levels up to 100 mg/kg on spontaneous locomotion or one measure of pain response (acetic acid-induced writhing test). Body temperature was unaffected in rats up to a dose of 100 mg/kg.

Cardiovascular effects:

A conscious dog study [M-11-00106.001A] was conducted in 4 dogs in which TAK-375 was administered orally at a single dose level, 66 mg/kg and heart rate, blood pressure and the ECG was monitored for significant changes indicative of a treatment-related effect. Measurements were taken at -15 minutes, 0 minutes (drug administration) and 15, 30, 60, 120, 180, 240 and 360 minutes post-drug administration. Control animals were monitored similarly but received 0.9% saline. Exposure to TAK-375 was verified by toxicokinetic analysis of plasma samples at all post-drug observation time-points. TAK-375 administration was associated with a moderate increase in HR and mildly increased mean blood pressure mostly due to a slight increase in diastolic pressure observed 30 and 60 minutes after drug administration. No alteration of ECG waveforms was observed at any time-point. Peak levels of TAK-375 were observed at the 1 hr measurement with high levels of TAK-375 maintained throughout the 0.5 hr – 2 hr post-administration period. T_{max} occurred at 1.3 hr with the average $AUC_{0-6\text{ hr}}$ being 35.3 $\mu\text{g}\cdot\text{h/mL}$. The level of M-II was not quantified in this assay nor, obviously, the time to peak levels of this metabolite.

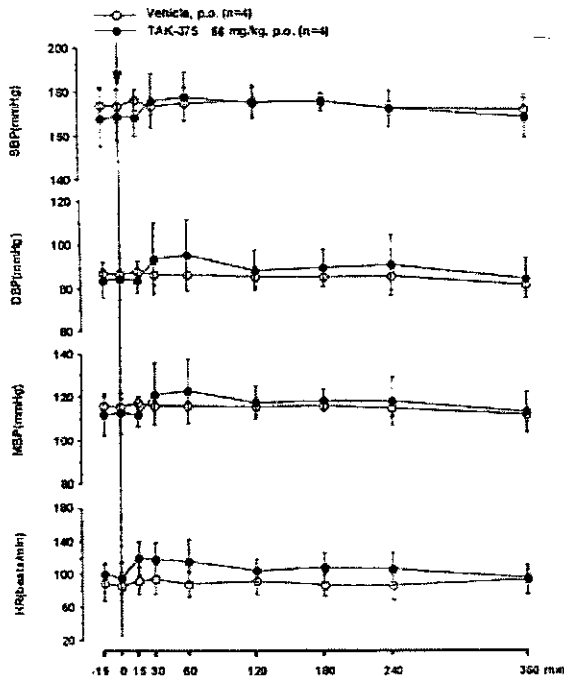


Fig. 10 Effects of TAK-375 on systolic (SBP), diastolic (DBP) and mean (MBP) blood pressure and heart rate (HR) in conscious dogs

TAK-375 was subsequently studied in an *in vitro* sheep purkinje fiber assay. TAK-375 or the positive control dl-sotalol was dissolved in DMSO (0.1 vol%) with final perfusion concentrations of 1, 10 and 100 μM for TAK-375 and 30 μM for sotalol. Four fibers were subjected to the test articles while 4 were exposed to vehicle only. As can be seen in the Sponsor’s table below, TAK-375 had no apparent effect on the shape of the purkinje fiber action potential at concentrations below 10 μM but at the high concentration of 100 μM there appeared to be a shortening of the action potential duration at 60% and 90% repolarization. Noted at this concentration also was a decreased upstroke amplitude and slightly depolarized diastolic membrane potential which may explain both the reduction in upstroke amplitude and shortened action potential. Rate of upstroke was, however unaffected at all concentrations.

	TAK-375 / Vehicle Concentration		
	Mean difference between groups (± s.d. mean difference) [†]		
	1 μmol/L / 0.1vol%	10 μmol/L / 0.1vol%	100 μmol/L / 0.1vol%
APD ₅₀ (%)	+1.9 ± 18.0	+6.5 ± 20.2	+10.9 ± 54.3
APD ₆₀ (%)	-1.8 ± 4.4	-8.9 ± 8.2	-50.1 ± 9.9***
APD ₉₀ (%)	-1.4 ± 3.5	-6.6 ± 6.7	-39.6 ± 8.6***
V _{max} (%)	+12.2 ± 15.3	+18.6 ± 18.2	+0.3 ± 19.1
UA (mV)	+0.5 ± 2.5	-0.5 ± 2.8	-12.8 ± 6.7*
DMP (mV)	-1.5 ± 2.7	+3.5 ± 5.4	+5.3 ± 3.4*

[†] Mean differences are relative to the vehicle group. *P < 0.05, ***P < 0.001

Thus, concentrations of TAK-375 which reach 100 μM may affect cardiac action potential characteristics though an effect on ion channels located on purkinje cells. *No Purkinje fiber study has been conducted with M-II, however.*

TAK-375 as well as M-II was further examined for effects on a critical outward rapid outward delayed-rectifying potassium current, I_{Kr} , in the hERG channel assay [M-11-00557 and M-11-00562]. These GLP-studies were conducted in HEK293 cells stably transfected with the human *ether-a-go-go* related gene and utilized a whole cell patch clamp method. Vehicle (DMSO), TAK-375 and M-II were evaluated at perfusion concentrations of 10^{-6} , 10^{-5} and 10^{-4} M while the positive control E-4031 was perfused at a concentration of 10^{-7} in these assays. Results indicated neither TAK-375 nor the active metabolite M-II was a direct hERG channel blocker. The residual current remaining after cells were exposed to vehicle alone was 84-87% of the baseline current. The residual current remaining after exposure to TAK-375 at 10^{-6} , 10^{-5} and 10^{-4} M was 94.2, 89.4 and 81.3% of baseline for a control-corrected maximal inhibition of 6.9%. Residual current remaining after M-II exposure at identical concentrations was 92.1, 85.0 and 83.9% for a control-corrected maximal inhibition of 0.4%. Assay sensitivity was demonstrated with E-4031 producing approximately 90% inhibition of baseline current when used at 10^{-7} M.

An *in vivo* study of the effect of TAK-375 on body temperature, heart rate, mean blood pressure and spontaneous locomotor activity in cynomolgus monkeys was conducted [M-11-95] using an escalating dose paradigm initially and then followed by a 5-day repeated dose experiment at a single dose level. For the first part of the experiment, TAK-375 was administered by oral gavage at a dose of 0 (vehicle, 0.5 w/v% methylcellulose), 2, 20, or 200 mg/kg to 3 male monkeys in the morning with a washout of 1-6 days between doses. No effects were observed at the 2 or 20 mg/kg p.o. dose level on any measured parameter after morning administration when measured at the morning measurement. However at 200 mg/kg ↓HR (-20%), ↑MBP (+13%) and ↓activity (-67%) were observed in treated animals at the morning observation time when compared with these animals when given vehicle alone. A mild drop in body temperature was also observed. These effects were observed to last 6-8 hr but by evening assessment all values were indistinguishable from evening values after vehicle treatment with the exception of a maintained ↑MBP (+18%). The 200 mg/kg TAK-375 dose was therefore selected for the follow-up 5-day repeated administration experiment to determine if these responses were durable. The results of this study can be seen in Sponsor's table reproduced below.

The results of 5-day repeated administration are shown in Table 3 and summarized below.

	200 (mg/kg/day)			
	Non-treatment	1st dose	3rd doses	5th doses
BT (°C)	37.06±0.11	36.50±0.25	36.44±0.23	36.69±0.26
HR (bpm)	155±7	113±23	119±23	126±24
MBP (mmHg)	79±1	84±5	84±4	83±6
ACT (counts/2 min)	1788±522	693±466	405±421	719±514

Each data represents the mean with SD of 3 animals during the 6-hour period after dosing or from 9:00 to 15:00 (non-treatment).

Repeated dosing of monkeys with 200 mg/kg/day TAK-375 for 5 days replicated the previously observed decrease in body temperature, heart rate and activity levels while demonstrating the increase in mean blood pressure. Tolerance to these effects did not appear within the 5 days of TAK-375 administration.

Pulmonary effects:

Respiratory responses were also monitored in the above-mentioned conscious dog study [M-11-00106.001A]. No effects were observed at the single dose examined, 66 mg/kg.

Renal effects:

Rats were administered vehicle (saline), HCTZ 10 mg/kg or TAK-375 at doses of 10, 30 or 100 mg/kg by oral gavage and resulting effects on urinary output was evaluated. No alteration of urine volume, urinary sodium or urinary potassium concentration was observed in rats treated with TAK-375 at any dose level when compared with vehicle treated rats. In contrast, HCTZ significantly increased urinary volume, sodium and potassium concentration as expected.

Gastrointestinal effects:

Interaction with the autonomic system and smooth muscle activity is considered limited except at high concentration (10^{-4} M) in which a slight inhibition of spontaneous motility as well as contractions induced by acetylcholine and histamine were observed *in vitro* in isolated rabbit and guinea pig ileum, respectively.

TAK-375 associated effect on the GI system was tested in rats in models of both gastric emptying (n= 6/group) and intestinal motility (n = 6/group) with doses up to 100 mg/kg p.o. Mild effects of test article on gastric emptying was constrained to the 100 mg/kg group and consisted of an inhibition that was approximately 13% below control value. A slight increase in intestinal motility as measured by meal transport was, however, observed at the 100 mg/kg dose level (+8% above control). No effects on meal distance traveled were observed at 10 or 30 mg/kg p.o. dose levels.

Abuse liability:

Male F344/DuCrj rats administered vehicle or TAK-375 in doses of 200 or 600 mg/kg or the positive control substance Diazepam at 300 mg/kg for 4 weeks followed by 1 week withdrawal period. During the dosing period rats administered TAK-375 demonstrated decreased bw and decreased fc. During withdrawal period no clinical signs were noted and body weight increased relative to the final pre-withdrawal value while fc was unchanged compared with controls. This was in contrast to the positive control drug diazepam in which withdrawal was associated with a transient decrease in feces and evidence of irritability, weight loss and decreased food intake. Mean TAK-375 plasma concentration at the high dose was 90 ng/mL while the mean M-II plasma concentration was 40 ng/mL. *The Sponsor's conclusion is that TAK-375 showed no physical dependence potential in rats, a finding with which I am in agreement.*

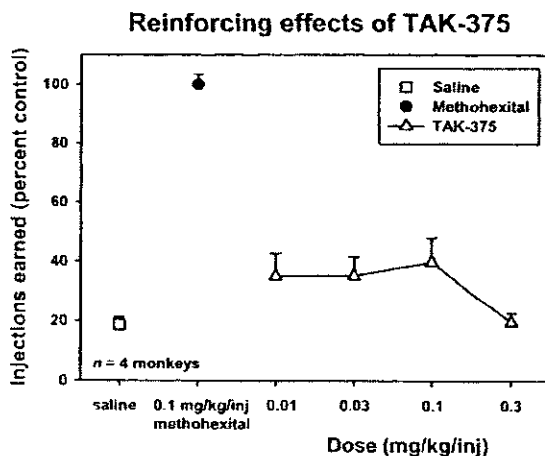
A subsequent physical dependence study was conducted in rhesus monkeys (3 male, 1 female) in which TAK-375 was administered through an in-dwelling catheter at a dose of 10 mg/kg/day in 0.5 (w/v%) methylcellulose for 52 weeks. This dose was selected from previous shorter studies in rhesus monkeys which showed unacceptable toxicity at 20 mg/kg/day and higher. The study design included 5-day drug discontinuation periods during Weeks 14, 27, 40 and 53 during which time only vehicle was administered. Monkeys were administered drug or vehicle 15 minutes prior to assessment of operant conditioning response

and other monitored parameters included body weight and general behavior. Animals were trained on a fixed ratio response procedure to obtain food reward or avoid aversive foot shock under cues of different color light. Concentration of TAK-375 in plasma was assessed during Week 4 at several time-points following drug administration as well as a pre-dose assessment. Blood was also sampled for TAK-375 concentrations every day during the 5-day drug discontinuation period as well as one day prior to discontinuation and one day after reinstatement of the dosing regimen to ensure adequate reductions in prevailing blood concentrations to support the interpretation of drug withdrawal potential. Note that M-II, the active metabolite observed to be the principal component in the human plasma, was not assessed in plasma and would not be expected to be present in a significant concentration if previous metabolism studies in the monkey are correct. Nevertheless, the nearly identical pharmacology of the metabolite would suggest little possibility of a significant pharmacodynamic difference between the parent and metabolite. Results of the toxicokinetic segment of the study revealed adequate exposure of monkeys to TAK-375 though the single female in the study had markedly higher plasma concentration and exposure ($C_{max} \sim 57$ to 164 ng/mL [σ], 454 ng/mL [ρ]; $AUC_{0-24} \sim 254$ to 382 ng•h/mL [σ], 3035 ng•h/mL [ρ]). Exposure did not appear to decrease substantially with repeated dosing. Periods of drug discontinuation were associated with falls in plasma TAK-375 concentrations to below detectable levels (0.5 ng/mL) in males though in the single female small levels of TAK-375 were sporadically detected but were significantly lower than during the dosing period. No mortality was observed during the 52 week study. No changes in body weight were observed. No changes in clinical signs were noted during the study or during the periods of drug discontinuation relative to dosing period. No increase in motor activity observed during periods of drug discontinuation. The female did have occasional bouts of seizures beginning in the 9th month of the study and going on until the 10th month but these appeared to resolve, did not appear to be associated with drug discontinuation periods or drug administration periods consistently though the specific time of the seizure relative to drug administration and TAK-375 blood levels were not determined by the study director. Nevertheless, seizure activity appeared to be reduced over the succeeding two months and was not observed near the end of the study. Behavioral responses to fixed dose conditioning for a food reward were not affected by the status of drug administration/drug discontinuation though the female rhesus had an apparent decrease in food response rate over the 52-week study. The response to fixed ratio shock condition was unchanged during the study in both males and females and was not altered during the drug discontinuation period. Overall, no changes were seen in any animal to occur during the period of drug discontinuation in a manner that would indicate the potential for a TAK-375 withdrawal response.

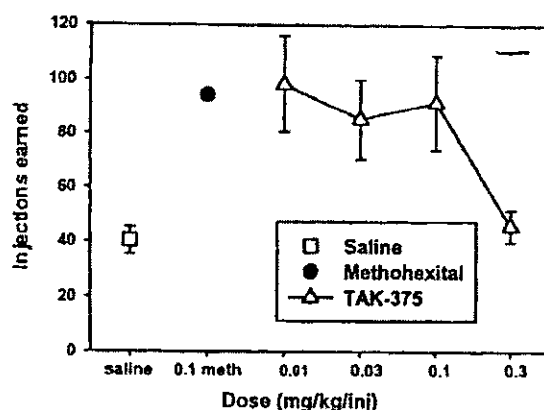
The potential for benzodiazepine-like effects was explored in monkeys in two drug discrimination studies [01-02-TL-375-011 and 01-02-TL-375-012]. TAK-375 was administered in ascending doses from $0.32 - 10.0$ mg/kg i.v. to determine if monkeys could discriminate the test article from midazolam, a drug they were previously trained to discriminate from vehicle. TAK-375-responding resembled vehicle responding at all doses and in all animals. In the second study, animals were made dependent to diazepam and trained to discriminate low doses of the benzodiazepine antagonist flumazenil. Animals were administered TAK-375 i.v. in doses between 0.32 and 10.0 mg/kg 15 minutes prior to the testing session to determine if the dose-response curve for flumazenil would be shifted

rightward which would indicate lessened sensitivity to the benzodiazepine-antagonist effects and thus a potential for benzodiazepine agonist pharmacodynamic response. No such response was observed and the Sponsor concluded that TAK-375 produces no benzodiazepine-like discriminative stimulus. Despite the lack of a positive control in these studies this reviewer concurs with the evidence that TAK-375 is unlikely to possess benzodiazepine-like subjective effects.

The potential rewarding/reinforcing effects of TAK-375 was studied in rats and rhesus monkeys using conditioned place preference (rats) and self-administration (monkey) paradigms. Administration of TAK-375 at doses of 3, 10 and 30 mg/kg p.o. [M-11-00621.001R] to male Wistar rats did not appear to increase the duration of time spent in the drug-associated box, a finding which was similar to the absence of reward apparent for melatonin at higher doses of 10, 30 and 100 mg/kg p.o. The positive controls consisting of morphine (1 mg/kg), diazepam (5 mg/kg) and triazolam (0.5 mg/kg) were associated with significant place preference, however. In a study of the potential rewarding effect of self-administration of TAK-375 in rhesus monkeys [01-02-TL-375-010], four monkeys with a previous history of methohexital self-administration received a jugular, brachial or femoral catheter for intravenous administration of study drug which was dissolved in saline up to concentrations of 2 mg/mL. Note this would appear to be likely to exceed solubility limits for TAK-375 (~ 0.2 mg/mL in aqueous solutions) though no observation of precipitate was mentioned though formulation mistakes were noted upon assessment of dosing solutions by contract laboratory. Formulations were subsequently altered to provide the correct dose. Monkeys were evaluated in a substitution self-administration procedure with methohexital serving as the positive control and saline as the negative control.



When compared over the 4 animals tested, TAK-375 responding appeared to be more similar to the responding rate associated with saline than methohexital (above figure) though responding was not at the same level as the negative control. This slight elevation is due to one monkey (— figure next page) appearing to exhibit self-administration of TAK-375 at a rate which approximated the level of responding to methohexital.



The Sponsor notes that this animal also had a high level of saline:methohexital responding and as such may be pre-disposed to self-administer. No evidence in the monkey's background could be found to explain this higher rate of background and TAK-375 responding and in other drug self-administration studies this animal did not appear different from other monkeys.

However, in all monkeys there was no indication of a dose-response relationship to responding rates in the TAK-375 condition and in fact responding was lower at the highest dose tested (0.3 mg/kg i.v.). The sponsor concludes that:

"It is difficult to discern whether the results indicate that the compound might have abuse liability in this monkey or not under these circumstances. In the other three monkeys, no reinforcing effect was detected" (p.12, study 01-02-TL-375-010).

A subsequent study involving rhesus monkeys and i.v. administration of TAK-375 [M-11-00626] contained two separate experiments for two different purposes. The first experiment involved the assessment of gross behavioral observations to determine acute CNS effects after a single i.v. administration of 1 or 2 mg/kg TAK-375. Results indicated TAK-375 administration was not associated with any observable behavioral effect in monkeys under this condition. A subsequent analysis of the plasma concentrations of TAK-375 and metabolites when administered i.v. at a dose of either 0.2 or 0.8 mg/kg i.v. revealed $C_{5 \text{ min}}$ levels of TAK-375 were 249 ng/mL (0.2 mg/kg) and 1077 ng/mL (0.8 mg/kg) indicating a linear exposure. No evidence of the M-II metabolite (or M-III or M-IV) was observed up to 24 hr after TAK-375 administration. Low levels of M-I were detected. The second set of experiments involved the surgical placement of an in-dwelling catheter with intravenous access and the examination of self-administration potential of TAK-375 in either a Fixed ratio (FR:50) over 2 hr sessions for 4 days per dose level or continuous self-administration regimen for 2-4 weeks per dose level. Doses of TAK-375 tested were .025, .05, .1 or .2 mg/kg/infusion in the fixed ratio regimen and 0.25, .1, and 0.4 mg/kg/infusion in the continuous infusion regimen. Assay sensitivity was demonstrated in all studies with the exception of the continuous infusion regimen by use of a positive control solution of sodium pentobarbital. Under the conditions of the continuous infusion regimen, only 1 of 4 animals in the pentobarbital (positive) control group demonstrated self-administration.

Otherwise, in all animals and in both regimens, self-administration patterns of TAK-375 were not distinguishable from vehicle and differed markedly from the pattern of response to pentobarbital. Plasma exposure to TAK-375 at 0.2 mg/kg/i.v. infusion was characterized in the 1st part of this study and was linear through 0.8 mg/kg/i.v. infusion and indicated that monkeys were exposed to TAK-375 at plasma levels that were over 150-fold and 300-fold higher at 0.2 mg/kg/infusion and 0.4 mg/kg/infusion, respectively, than that expected with clinical exposure to the MRHD of 8 mg/day (~1.5 ng/mL).

2.6.2.5 Pharmacodynamic drug interactions

TAK-375 was evaluated in a rota-rod test in mice to determine if the compound would impair motor coordination when given alone and explore the potential for TAK-375 to exacerbate the impairment produced by the benzodiazepine diazepam. Mice were administered vehicle, TAK-375 3, 10 or 30 mg/kg, melatonin (3 – 30 mg/kg), N-acetyl-5-HT (3 - 30 mg/kg), or diazepam (3 – 10 mg/kg) by oral gavage prior to initiating the rota-rod test. TAK-375 at a dose up to 30 mg/kg p.o. did not have an appreciable effect on rota-rod performance, a finding which was similar to that observed with melatonin and N-actyl-5-HT up to equivalent doses. Diazepam administered at a dose of 5 and 10 mg/kg, though not at 3 mg/kg, produced demonstrable reductions in rota-rod performance. Co-administration of TAK-375 up to 30 mg/kg with diazepam 3 mg/kg – a diazepam dose which by itself does not produce rota-rod performance deficits – did not qualitatively or quantitatively alter the motor impairment. Confirming the validity of the study methods, melatonin and N-acetyl-5-HT at a dose of 3 and 10 mg/kg produced a significant augmentation of diazepam-induced reduction in rota-rod performance. The mechanism by which melatonin interacts with diazepam would therefore appear to be through a mechanism other than the ML-1 receptor as the more potent TAK-375 had no effect even at higher doses. Regardless, no interaction between TAK-375 and the benzodiazepine diazepam on a measure of motor coordination could be demonstrated under these conditions.

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2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.3.2A Primary Pharmacodynamics – In Vitro

Test Article: TAK-375

System Evaluated	Test System	Compounds Evaluated	Noteworthy Findings	GLP Compliance	Report No.
Mel _{1a} /Mel _{1c} receptor binding	Chick forebrain Mel _{1a} /Mel _{1c} receptors	TAK-375	K _i =23.1±0.336 pmol/L	No	M-11-00617
		Melatonin	K _i =368±8.76 pmol/L		
		2-iodomelatonin	K _i =24.8±1.73 pmol/L		
		N-acetyl-5-HT	K _i =583,000±22,000 pmol/L		
		Prazosin	K _i =2,360,000 pmol/L		
MT ₁ receptor binding	Syrian hamster brain MT ₁ receptors	TAK-375	K _i =2,650±183 nmol/L	No	M-11-00617
		Melatonin	K _i =24.1±0.536 nmol/L		
		2-iodomelatonin	K _i =0.964±0.0154 nmol/L		
		N-acetyl-5-HT	K _i =15.7±2.79 nmol/L		
		Prazosin	K _i =6.16±0.464 nmol/L		
MT ₁ receptor binding	CHO cells expressing human MT ₁ receptors	TAK-375	K _i =14.0±0.508 pmol/L	No	M-11-00617
		Melatonin	K _i =80.7±2.06 pmol/L		
		2-iodomelatonin	K _i =13.1±0.337 pmol/L		
		N-acetyl-5-HT	K _i =81,300±6,930 pmol/L		
		Prazosin	K _i =2,730,000 pmol/L		
Forskolin stimulated cAMP production	CHO cells expressing human MT ₁ receptors	TAK-375	IC ₅₀ =21.2±5.38 pmol/L	No	M-11-00617
		Melatonin	IC ₅₀ =77.8±14.6 pmol/L		
		2-iodomelatonin	IC ₅₀ =26.8±7.43 pmol/L		
MT ₁ receptor binding	CHO cells expressing human MT ₁ receptors	TAK-375	K _i =112±5.33 pmol/L	No	M-11-00618.001R
		M-II (2S,8S)	K _i =566±13.1 pmol/L		
		Melatonin	K _i =383±4.99 pmol/L		
		2-iodomelatonin	K _i =188±3.86 pmol/L		
		N-acetyl-5-HT	K _i =3,640,000±31,100 pmol/L		
		Prazosin	K _i =5,370,000 pmol/L		

Note: Values presented are means ± S.E.M.
CHO: Chinese hamster ovary.

2.6.3.2A Primary Pharmacodynamics – In Vitro

Test Article: TAK-375 (continued)

System Evaluated	Test System	Compounds Evaluated	Noteworthy Findings	GLP Compliance	Report No.
Forskolin stimulated cAMP production	CHO cells expressing human MT ₁ receptors	TAK-375	IC ₅₀ =53.4 (40.7-70.3) pmol/L (a)	No	M-11-00618.001R
		M-II (2S,8S)	IC ₅₀ =1,470 (930-2380) pmol/L (a)		
		Melatonin	IC ₅₀ =904 (714-1,150) pmol/L (a)		
		2-iodomelatonin	IC ₅₀ =60.7 (44.0-83.9) pmol/L (a)		
MT ₁ receptor binding	CHO cells expressing human MT ₁ receptors	TAK-375	K _i =11.6±0.866 pmol/L	No	M-11-00383.001R
		M-II (2S,8S)	K _i =114±12.5 pmol/L		
		Melatonin	K _i =45.4±4.11 pmol/L		
		2-iodomelatonin	K _i =19.9±1.23 pmol/L		
Mel _{1a} /Mel _{1c} receptor binding	Chick forebrain	TAK-375	K _i =19.9±1.23 pmol/L	No	M-11-00383.001R
		M-I	K _i =236,000 pmol/L		
		M-II	K _i =38,000±1280 pmol/L		
		M-IV (2S,8S)	K _i =236,000 pmol/L		
		M-V	K _i =4,940±97.5 pmol/L		
		TAK-375	K _i =21.4±2.23 pmol/L		
		M-II (2S,8S)	K _i =554±74.3 pmol/L		
		M-IV (2S,8S)	K _i =554±74.3 pmol/L		
MT ₁ receptor binding	Syrian hamster brain	TAK-375	K _i =4780±832 nmol/L	No	M-11-00383.001R
		M-I	K _i =9000 nmol/L		
		M-II (2S,8S)	K _i =9900 nmol/L		
		M-II	K _i =6,570±263 nmol/L		
		M-V	K _i =4,570±321 nmol/L		
		TAK-375	K _i =3,430±112 nmol/L		
		M-IV (2S,8S)	K _i =9,000 nmol/L		
		M-IV	K _i =9,000 nmol/L		

Note: Values presented are means ± S.E.M., except where indicated
CHO: Chinese hamster ovary.
(a) (95% confidence intervals)

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2.6.3.2A Primary Pharmacodynamics – In Vitro

Test Article: TAK-375 (continued)

System Evaluated	Test System	Compound Evaluated	Noteworthy Findings	GLP Compliance	Report No.
Forskolin stimulated cAMP production	CHO cells expressing human <i>h47₁</i> receptors	TAK-375	IC50 = 12.4 pmol/L	No	M-11-00353.001R
		M-II (2.5, 8.5)	IC50 = 208 pmol/L		
		Melatonin	IC50 = 48.1 pmol/L		
Forskolin stimulated cAMP production	Neonatal rat pituitary	TAK-375	0.001 nmol/L: 119.3% (a)	No	M-11-00619
			0.003 nmol/L: 107.1%		
			0.01 nmol/L: 127.7%		
			0.03 nmol/L: 65.41%		
			0.1 nmol/L: 31.86%		
		Melatonin	0.3 nmol/L: 32.51%		
			0.01 nmol/L: 112.4%		
			0.03 nmol/L: 91.93%		
			0.1 nmol/L: 25.56%		
			0.3 nmol/L: 3.884%		
1 nmol/L: 0.009%					

Note: Values presented are means ± S.E.M., except where indicated.

CHO: Chinese hamster ovary.

(a) Numbers represent percent of control value

2.6.3.2B Primary Pharmacodynamics – In Vivo

Test Article: TAK-375

System Evaluated	Species/Strain	Method of Admin.	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report No.
Effect on sleep promotion	Crib-raising macaques monkeys	Oral gavage	TAK-375: 0, 0.001, 0.03 and 0.3	4 F	TAK-375 (0.03-0.3 mg/kg): dose-dependent reductions in latency to sleep onset and increases in sleep duration. EEG activity similar to that in naturally occurring sleep. Melatonin (0.3-3 mg/kg) non-dose-dependent reductions in latency to sleep onset without effects on sleep duration. Zolpidem: minimal effects on latency to sleep onset. at 30 mg/kg induced apparent sedation and muscle relaxation, qualitative differences from naturally occurring EEG activity during sleep.	No	M-11-00752
			Melatonin: 0.03, 0.1, 0.3 and 10. 30				
Effect on sleep promotion	Crib-raising macaques monkeys	Oral gavage	M-II (2.5, 8.5): 0, 1, 3, and 10	6F	3 mg/kg significantly reduced latencies to onset of light sleep and slow wave sleep. 3 and 10 mg/kg trends for an increase in total sleep duration. All doses: no abnormal waveforms in EEG activity.	No	M-11-00736
Effect on sleep promotion	Cats	Oral gavage	TAK-375: 0, 0.0001, 0.001, 0.01, and 0.1	5M, 9F	TAK-375 (0.01-0.1 mg/kg): decreased wakefulness and increased slow wave sleep up to 6 hours post dose. At 0.1 mg/kg it also significantly increased REM duration and tended to reduce sleep latencies. Melatonin (1.0 mg/kg) decreased wakefulness and increased slow-wave sleep up to 2 hours post dose. Doses of 0.01 and 0.1 mg/kg increased slow wave sleep. Effects weaker than those with TAK-375. No trends for effects on sleep latencies.	No	M-11-00613
			Melatonin: 0.0001, 0.001, 0.1, and 1.0				
Effect on sleep promotion	Cats	Oral gavage	M-II (2.5, 8.5): 0, 0.001, 0.01, and 1.0	5M, 3F	M-II (0.1-1.0 mg/kg): significantly decreased wakefulness and increased slow-wave sleep for up to 6 hours post dose. M-II (0.01 mg/kg) significantly increased slow-wave sleep and tended to decrease wakefulness. M-II was 10 to 100-fold less potent than TAK-375.	No	M-11-00624.001R

REM: rapid eye movement sleep

2.6.3.3 Secondary Pharmacodynamics

Test Article: TAK-375

Organ System Evaluated	Species/Strain	Method of Admin.	Doses (mg/kg) (a)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report No.
Re-entrainment of running wheel activity	Wistar rats	Oral gavage	TAK-375: 0.1 and 1.0 mg/kg for 14 days. Melatonin: 1 and 10 mg/kg for 14 days.	5M	Groups treated with all doses of TAK-375 and 10 mg/kg melatonin showed more rapid re-entrainment of wheel running activity following an 8 hour phase shift when compared to vehicle control groups.	No	M-11-00323.001R
Learning and memory (Morris water maze)	Wistar rats	Oral gavage	TAK-375: 0, 0.1, 0.3, and 30. Melatonin: 0, 10, 30, and 100. Dizepam: 0, 3, 10, and 30. Trazolam: 0, 0.1, 0.3, and 1.	5M	TAK-375 no significant effects. Melatonin no significant effects. Dizepam and melatonin: dose-dependent increases in latencies to find submerged platform and reductions in number of crossings over the platform was on the probe trial.	No	M-11-00620
Learning and memory (Delayed matching to position)	F344 rats	Oral gavage	TAK-375: 0, 0.1, 0.3, and 30. Melatonin: 0, 10, 30, and 100. Dizepam: 0, 3, 10, and 30. Trazolam: 0, 0.1, 0.3, and 1.	9-16M	TAK-375 no significant effects. Melatonin no significant effects. Dizepam and melatonin: significant, dose-dependent decreases in the number of correct responses.	No	M-11-00625

(a) Single dose unless specified otherwise

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2.6.3.3 Secondary Pharmacodynamics

Test Article: TAK-375 (continued)

Organ/Systems Evaluated	Species/Strain	Method of Admin.	Doses (mg/kg) (a)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report No.
Nocturnal plasma melatonin levels	Wistar rats	Oral gavage	TAK-375: 0, 9.3 and 3.9	♂M	Groups treated with TAK-375 tended to show elevated melatonin levels 2-2.5h after treatment relative to controls. Groups treated with TAK-375 tended to show reduced melatonin levels 10-16.5h after treatment relative to controls. Effects were not statistically significant by ANOVA (P<0.05).	No	M-11-00623
Effects of 5-HT3 receptor antagonists on TAK-375-induced emesis	Cynomolgus monkeys	Oral gavage Intravenous	TAK-375: 260 Malatonin: 200 and 1000 Granisetron: 0.3 Luzindole: 40	3-5	TAK-375 and malatonin (1000 mg/kg) produced emesis in all animals. The vomiting induced by TAK-375 and malatonin was inhibited by granisetron. The vomiting due to TAK-375 was also prevented by luzindole.	No	M-11-00708

(a) Single dose unless specified otherwise.

2.6.3.4 Safety Pharmacology

Test Article: TAK-375

Organ Systems Evaluated	Species/Strain	Method of Admin.	Doses (a) (mg/kg) (b)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report No.
Effects on the Central Nervous System							
Behavior (Irwin test)	Mice	Oral gavage	0 and 100	♂M	100 mg/kg: slight sedation within 30 min post-dosing, not present at 60 min.	No	M-11-00106.001A
Spontaneous locomotor activity, electroshock-induced seizures, acetic acid induced writhing	Mice	Oral gavage	0, 10, 30, and 100	10M	100 mg/kg: prevention of electroshock-induced tonic extensor seizures.	No	M-11-00106.001A
Rota-rod performance	Mice	Oral gavage	0, 3, 10, and 30	12M	None	No	M-11-00622
Pentobarbital-induced sleep time, body temperature	Rats	Oral gavage	0, 10, 30, and 100	♂M	100 mg/kg: ↑ sleep time	No	M-11-00106.001A
Behavioral observations	Monkeys	Intravenous	0, 1, and 2	2 dose (total 3M, 3F)	None	Yes	M-11-00626
Electro-encephalogram	Cats	Oral gavage	10	3M, 3F	↑ drowsiness, ↓ arousal, no abnormal EEG waveforms.	No	M-11-00106.001A
Effects on the Respiratory and Cardiovascular Systems							
Isolated Purkinje fibers - action potential parameters	Sheep	In vitro	1, 10, and 100 μmol/L	-	1 and 10 μmol/L: No effects 100 μmol/L: ↓ APD60 & 93, ↓ upstroke amplitude, small depolarization of the diastolic membrane potential.	Yes	M-11-102
hERG current: TAK-375	HEK-293 cells expressing hERG	In vitro	1, 10, and 100 μmol/L	-	None	Yes	M-11-00557
hERG current: M-II (2N k7)	HEK-293 cells expressing hERG	In vitro	1, 10, and 100 μmol/L	-	None	Yes	M-11-00362
Heart rate, blood pressure, electro-cardiogram, respiration	Beagle dogs	Oral gavage	0 and 66	4M	None	No	M-11-00106.001A

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Organ Systems Evaluated	Species/Strain	Method of Admin.	Doses (a) (mg/kg) (b)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report No.
Heart rate, blood pressure, activity and body temperature	Cynomolgus monkeys	Oral gavage: AM or PM (c)	0, 2, 30 and 200	3M	AM: 200 mg/kg: w body temperature, heart rate, and activity; ↑ mean blood pressure. PM: 200 mg/kg: ↑ mean blood pressure.	No	M-11-93
Heart rate, blood pressure, activity and body temperature	Cynomolgus monkeys	Oral gavage	200 (d)	3M	↓ body temperature, heart rate, and activity; ↑ mean blood pressure.	No	M-11-93
Effects on the Autonomic Nervous System and Smooth Muscle							
Spasmodic-induced contractions in the isolated ileum	Cricetus puz.	In vitro	1, 10, and 100 μmol/L	-	100 μmol/L: slight inhibition of maximum contraction by acetylcholine and histamine.	No	M-11-00106.001A
Spontaneous motility in the isolated ileum	Rabbits	In vitro	10, 30, and 100 μmol/L	-	100 μmol/L: slight inhibition of spontaneous motility.	No	M-11-00106.001A
Effects on the Gastrointestinal System and Water and Electrolyte Metabolism							
Gastric emptying, intestinal transport, urine volume, urine sodium and potassium excretion.	Rats	Oral gavage	0, 10, 30, and 100	6M	100 mg/kg: significantly enhanced transport of a semi-solid meal by 8.1% without an effect on gastric emptying. No effect on urine volume or urinary electrolyte excretion.	No	M-11-00106.001A
Other Studies							
TAK-375 receptor binding and enzyme activity	Various receptors and enzymes	In vitro	Binding: 10 μmol/L Enzyme activity: 10-1000 μmol/L	-	TAK-375: none Mefenazin: 5-HT _{1A} receptor, K _i =5.6 μmol/L	No	M-11-00618
TAK-375 M-II (2S,8S) receptor binding and enzyme activity	Various receptors and enzymes	In vitro	Binding: 10 μmol/L Enzyme activity: 10-1000 μmol/L	-	M-II (2S,8S): 5-HT _{2B} receptor, K _i =1.73 μmol/L	No	M-11-00389

Organ Systems Evaluated	Species/Strain	Method of Admin.	Doses (a) (mg/kg) (b)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report No.
Physical dependence	F344 rats	Diet	TAK-375: 0, 200, and 600 (a) Diazepam: 300 (a)	TAK-375: 14M (f) Diazepam: 103M	TAK-375: no physical dependence noted after cessation of treatment Diazepam: signs of physical dependence after cessation of treatment (irritability, decreases in food, body weight, and food consumption).	Yes	M-11-00168.001
Physical dependence	Monkeys	Intragastric	10	3M, 1F	No withdrawal signs were evident.	No	01-02-TL-575-013
Abuse liability: Conditioned place preference	Wistar rats	Oral gavage (g)	TAK-375: 3, 10 and 30 Mefenazin: 10, 30 and 100 Morphine: 1 Tiazepam: 0.5 Diazepam: 5	7-11M	Animals treated with TAK-375 or mefenazin at any dose level failed to establish a conditioned place preference. Animals treated with morphine, mefenazin or diazepam demonstrated statistically significant place conditioning.	No	M-11-00622.001R
Abuse liability: Limited access self-administration	Rhesus monkeys	Intravenous (h)	TAK-375: 0, 0.01, 0.03, 0.1, and 0.3 Methohexital cadmate: 0.1 (h)	4	No reinforcing effect was seen in 3 of 4 animals. One animal demonstrated non-dose dependent ~ in TAK-375 self-administration.	No	01-02-TL-375-010
Abuse liability: Limited access self-administration	Rhesus monkeys	Intravenous (h)	TAK-375: 0.025, 0.05, 0.1, 0.2, and 3 → (i) Pantobarbital sodium: 0.125, 0.25, 0.5, and 1.0 (i)	2M, 2F	TAK-375: number of self-administrations was similar to the vehicle control. Pantobarbital: number of self-administrations greater than vehicle control.	Yes	M-11-00626

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Organ Systems Evaluated	Species/Strain	Method of Admin.	Doses (a) (mg/kg) (b)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report No.
Abuse liability: Common access self-administration	Rhesus monkeys	Intravenous (a)	TAK-375: 0.025, 0.1, and 0.4 (f)	2M, 2F	Number of self-administrations was similar to the vehicle control.	Yes	M-11-90626
Drug discrimination for benzodiazepine agonist-like effects	Rhesus monkeys	Intravenous (a)	TAK-375: 0.32, 1.0, 3.2, 5.6, and 10	4F	No evidence to support benzodiazepine-like discriminative stimulus effects in midazolam-discriminating monkeys.	No	01-02-TL-375-011
Drug discrimination for benzodiazepine agonist-like effects in the context of precipitated drug withdrawal	Rhesus monkeys	Intravenous Drinking water Subcutaneous	TAK-375: 1.0, 3.2, 5.6, and 10.0 Diazepam: 5.6 mg/kg/day Flumazenil: 0.0032 to 0.32	3M, 1F	No evidence to support benzodiazepine agonist-like discriminative stimulus effects of TAK-375 in diazepam-dependent monkeys after abrupt withdrawal by flumazenil.	No	01-02-TL-375-012

hERG indicates human *ether-a-go-go* related gene.

HEK indicates human embryonic kidney.

(a) Single dose unless specified otherwise.

(b) mg/kg unless specified otherwise.

(c) Dosing occurred at either 900 or 1700

(d) Dosing occurred at 0900 for 5 days

(e) Presented in powdered diet for 4 weeks, followed by a 1-week withdrawal period.

(f) Four additional animals were used as satellite animals for toxicokinetic analysis.

(g) Test article administered on Days 1 and 3 of conditioning; saline administered on Days 2 and 4

(h) Dose expressed as mg/kg infusion.

(i) Each dose available for self-administration 2 hours/day.

(j) Each dose available for self-administration 24 hours/day for 2-4 weeks.

2.6.3.5 Pharmacodynamic Drug Interactions

Test Article: TAK-375

Organ Systems Evaluated	Species/Strain	Method of Admin.	Doses (mg/kg) (a)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report No.
Diazepam-induced impairment of rota-rod performance	Mouse	Oral gavage	Diazepam: 0, 3, 5 and 10 TAK-375: 0, 3, 10 and 30 Malonin: 0, 3, 10 and 30 N-acetyl-5-HT: 0, 3, 10 and 30 Diazepam: 3 TAK-375: 0, 3, 10, and 30 Malonin: 0, 1, 3 and 10 N-acetyl-5-HT: 0, 1, 3, and 10	12M	Diazepam produced significant impairments at 5 and 10 mg/kg. TAK-375, malonin and N-acetyl-5-HT had no effect on rota-rod performance when tested at doses of 3, 10 and 30 mg/kg. In animals pretreated with diazepam at 3 mg/kg, rota-rod performance was impaired after treatment with malonin (3 and 10 mg/kg) and N-acetyl-5-HT (3 and 10 mg/kg) but was not affected after treatment with TAK-375.	No	M-11-06623

(a) Single dose unless specified otherwise

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Nonclinical pharmacokinetic studies were conducted in rats, dogs and monkeys *in vivo* and *in situ*, and in *in vitro* cell lines to examine the characteristics of TAK-375. The key findings from these studies can be summarized by the following observations:

- TAK-375 is efficiently and quickly absorbed from the GI tract without preference as to location. Absorption through the GI tract is believed to be passive and due to the physiochemical properties of TAK-375 as suggested by its octanol/water coefficient *in vitro* studies in Caco-2 cells and the lack of interaction with the P-glycoprotein transporter. T_{max} is observed within the first half-hour of oral administration in all nonclinical species tested. Although well absorbed, bioavailability is low to very low in all species due to extensive first pass metabolism. Bioavailability in rats, monkeys and humans was calculated as: 6.3%, 0.3%, and 1.8%, respectively.
- Single-dose intravenous studies using [¹⁴C]TAK-375 indicate a short half-life in all nonclinical species tested. The $t^{1/2}$ in rat was judged to be 0.27 hr while the monkey was 0.82 hr. Subsequent human studies suggest the $t^{1/2}$ to be approximately 1.8 hr.
- Repeat-dose toxicokinetics were conducted in mice and rats in carcinogenicity studies, rats and monkeys in chronic toxicity studies, and in rats and pregnant rabbits

in reproductive toxicology studies. Gender differences were observed in rats such that exposure was higher in females than in males at dose levels tested. No such effects of gender were observed in mice, rabbits or monkeys. In general, exposure to TAK-375 (C_{max} and AUC) increased in a dose-proportional manner in rats until reaching dose levels of 1200 mg/kg, in mice until 300 mg/kg, rabbits until 300 mg/kg and in monkeys C_{max} was dose-proportional up to 200 mg/kg/day while AUC_{0-24} was linear but greater than dose-proportional. At higher doses a less than dose-proportional increase in exposure was observed with increasing dose.

- Repeated administration of TAK-375 in general and reproductive toxicology studies was associated with reduced exposure over time in the mouse at dose levels ≥ 100 mg/kg/day, rat at dose levels ≥ 150 mg/kg/day and rabbit at dose levels = 300 mg/kg/day but not in the monkey up to dose levels of 200 mg/kg/day. The reduction in exposure is considered a result of hepatic enzyme induction, discussed below.
- Distribution of radiolabel after administration of [^{14}C]TAK-375 was wide and rapid in rats, achieving maximal concentrations in most tissues within 30 minutes of drug administration. Other than the GI tract, the organs receiving the highest concentration of radiolabel were the liver, kidney, adrenal gland, and pituitary gland. Radioactivity rapidly decreased after 2 hr and was nearly absent in most tissues at 48 hr. These studies measured TAK-375-related compounds (i.e. TAK-375 and metabolites). The $t^{1/2}\alpha$ of TAK-375-related radioactivity was considered to be 1-3 hr while $t^{1/2}\beta$ was determined to be < 24 hr except in epididymal fat where it was nearly 60 hr. Overall, however, no evidence of significant tissue accumulation or retention was suggested. Oral administration of TAK-375 to rats with — ; analysis of TAK-375 and the active metabolite M-II content in plasma and brain demonstrated that both parent and metabolite penetrated into the CNS rapidly and exhibited similar concentrations in brain as found circulating in the plasma with TAK-375 concentrations roughly twice that of M-II. TAK-375-related compounds were found to cross the placenta and concentrate in the fetal plasma at a concentration nearly twice that of prevailing maternal plasma for up to 4 hr after which it resembled maternal concentrations. A subsequent study demonstrated both TAK-375 and M-II in fetal plasma after oral administration of TAK-375 to the pregnant dam. TAK-375 and/or metabolites appears to cross into breast milk rapidly as TAK-375-related radioactivity was detected in the milk of lactating rats with a peak 15 minutes after oral administration of [^{14}C]TAK-375. Plasma protein binding is moderately low in rat, monkey and human plasma being between 75 – 83% protein-bound *in vitro*.
- TAK-375 is extensively metabolized by the liver as determined in *in vivo* studies in rats and monkeys, *in situ* studies in rats and *in vitro* studies with hepatic microsomes. Metabolites identified in the plasma and excreta of the rat were M-I, M-II, M-III, M-IV, M-V, M-VI, M-VII, and M-VIII. M-IX was detected in the bile of the rat. Studies in monkey found these metabolites except M-V and M-IX were not found and M-VI and M-VIII were only detected in the urine. The primary circulating species in the plasma of the rat was M-III followed by M-I and M-VI. The major pathways of metabolism involved the hydroxylation of the propionamide side chain, forming M-II, and the oxidation of the ring hydroxyl group to form a carbonyl group, M-IV. Another major pathway involved cleavage of the dihydrofuran ring to form M-I. Other pathways typically involved ring or side chain oxidation. The only metabolite

formed in human that was not observed in nonclinical species was M-VIII-glucuronide. Of importance, M-II is known to be an active metabolite with similar pharmacologic activity to TAK-375 though possessing about 1/10th the potency at MT1 receptors.

- Studies to examine the CYP isoforms involved in TAK-375 metabolism were conducted with incubation of [¹⁴C]TAK-375 with hepatic microsomal preparations from rat, mouse, dog, monkey and human liver as well as studies with human and insect cells transfected with specific CYP isoforms. These studies demonstrated that the principal isoforms involved in TAK-375 metabolism were CYP1A2, CYP2C9 and CYP3A4. CYP1A2 is associated with formation of the M-II metabolite and CYP3A4 and to a lesser extent 2C9 are responsible for metabolism of the M-II metabolite as well as formation of the M-III and M-V metabolites. Interference with these enzymes in clinical drug-drug interaction studies (*described in the clinical pharmacology review*) using concomitant treatment with drugs that inhibit these metabolizing enzymes was documented to produce significant increases in plasma AUC of TAK-375: up to 190-fold with CYP1A2 inhibition using fluvoxamine, less so with other 1A2 inhibitors while 3A4 inhibition with ketoconazole moderately increased exposure to TAK-375 (1.8-fold). Inhibition of CYP2C9 with fluconazole increased TAK-375 exposure only slightly (1.5-fold).
- TAK-375 itself does not appear to be an inhibitor of cytochrome P450 enzymes up to concentrations of 10 µM when tested in cells containing recombinant CYP isoforms. IC₅₀ values were estimated to be between 10 – 100 µM for CYP2C8, 2C19 and 3A4 and higher for all other isoforms. M-II exhibited little inhibition of CYP isoforms with IC₅₀ values ≥ 100 µM. Direct evaluation of hepatic drug metabolizing enzymes in mice and rats was examined after 1 week of oral administration of TAK-375. Repeated administration of TAK-375 was associated with increased liver weight in mice with doses ≥ 300 mg/kg/day and in rats with doses ≥ 250 mg/kg/day. Analysis further demonstrated that a significant and dose-dependent increase in overall cytochrome P450 contents was achieved with doses ≥ 100 mg/kg/day in mice with marked induction at 1000 mg/kg/day and in rats at doses ≥ 250 mg/kg/day with marked induction at 1000 mg/kg/day though individual enzymes could be shown to be increased mildly though significantly at levels of 30 and 15 mg/kg/day, respectively. Generally, the pattern and degree of induction at the higher dose levels resembled the positive control group phenobarbital.
- Excretion studies in the rat using [¹⁴C]TAK-375 indicated radioactivity was principally excreted in the urine (58% within 24 hr) with fecal routes exhibiting a less prominent role (29% radioactivity excreted within 24 hr) and little (4.5%) through the respiratory route. Overall, 91% of administered radioactivity was recovered within 24 hr, 95% within 72 hr, and 95.5% within 168 hr. Recovery of radiolabel after intravenous administration of [¹⁴C]TAK-375 was nearly identical to the pattern seen with oral administration with recovery of radioactivity at 72 hr from urinary, fecal and respiratory routes being 59%, 32% and 5.5%, respectively. A study conducted in a bile-duct cannulated rats orally administered [¹⁴C]TAK-375 demonstrated that the great majority of radioactivity that was not excreted in the urine within 24 hr was found in the bile while low concentrations of radioactivity were observed in the feces. An additional study with bile-containing radiolabel demonstrated that 40% of

radioactivity associated with TAK-375-related compounds found in bile when placed intraduodenally is reabsorbed implying significant enterohepatic recirculation exists. Analysis of the excreta of rats and monkeys revealed that <1% of total detected radioactivity exists as unchanged TAK-375 in the urine, feces or bile of the rat or in the urine or feces of the monkey indicating that in these nonclinical species, the major pathway for elimination is through hepatic metabolism.

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

Studies of TAK-375 absorption were conducted in rats, dogs and monkeys as well as in the *in vitro* Caco-2 gut epithelial transport model. Many of these early studies utilized non-validated assay methods which failed to distinguish between TAK-375 and its metabolites one of which, M-II, is pharmacologically active. Due to this confound the primary and general observations which may be drawn from these absorption studies will be described without detailing some of the specific findings.

An initial study involving separate injection administration of [¹⁴C]TAK-375 to isolated stomach or different segments of the intestines revealed the majority of orally administered drug is likely to be absorbed during transit through the intestines with relative equal absorption in all segments. In all cases, administration of the radioactive test article to each segment of the intestines produced a higher C_{max} value and a shorter T_{max} latency than administration to the stomach [M-11-00748]. Further examination of absorption involved the administration of [¹⁴C]TAK-375 into the duodenal loop with cannulation of the portal vein from which blood samples were drawn [M-11-00749]. Data indicated that most of the dose (~75%) was absorbed within the first hour and 91% appeared in the plasma within 2 hr. which further indicates a high degree of absorption through the intestinal tract in the rat. Additionally, TAK-375 was not a substrate for the P-glycoprotein (PgP) transporter [M-11-00750]

[¹⁴C]TAK-375 was readily absorbed in studies conducted in the rat with maximum plasma concentrations occurring within 15 min of oral administration [M-11-00778]. Bioavailability of TAK-375 was low (~ 6.3%) due a significant degree of hepatic first pass metabolism. Females generally had higher exposures than males throughout the dosing range used in toxicity studies. Administration of [¹⁴C]TAK-375 to male dogs [M-11-00642] demonstrated even faster absorption as defined as latency to T_{max} (0.1 hr). TAK-375 was eliminated rapidly with an estimated t^{1/2} between 2-3 hr. As with the other nonclinical species tested, the cynomolgus monkey showed a rapid absorption of TAK-375 with an even shorter t^{1/2} of ~ 8 hr [M-11-782]. Of all species tested, including human subjects – with an oral bioavailability of 1.3% - bioavailability was lowest in the monkey (0.3%) due to marked first pass metabolism of the parent compound. Absorption appeared to be dose-proportional in general in this species.

Repeat-dose toxicokinetics of TAK-375 was examined as part of the nonclinical toxicology program and revealed that rodents and rabbits had significant reduction of exposure to TAK-375 with repeated exposure to the drug. This reduction was demonstrated to be due to a significant induction of hepatic metabolizing enzymes in subsequent studies.

No evidence of enzyme induction was found in studies of the monkey and in agreement with this finding no reduction in exposure was observed in repeat-dose toxicology studies. Gender differences in exposure to TAK-375 were only observed in rats and were not noted in mice or monkeys. Generally, exposure to TAK-375 was dose-proportional at low to moderate dose levels while negative departure from linearity was observed as the TAK-375 dose was increased.

The Sponsor summarized the pharmacokinetic values describing absorption, distribution and elimination after single dose oral or intravenous administration of TAK-375 in the following two tables:

Table 3.a Pharmacokinetics of Ramelteon Across Species Following Oral Administration

Species	Sex	Dose (mg/kg)	Formulation	T _{max} (hr)	C _{max} (ng/mL)	AUC (ng·hr/mL)	BA (%)	Report Number [Reference]
Rat	M	1	Susp(a)	0.25	17.5	21.3(b)	6.3	M-11-00781 [75]
Dog	M	1	Susp(a)	0.1	112	129 (c)	-	M-11-00642 [80]
Monkey	M	0.3	Susp(a)	0.33	0.525	0.414(b)	0.3	M-11-00782 [76]
Human	M	-0.23(d)	Tablet	0.75	3.89	0.325(b)	1.77(e)	TAK-375/EC003 [77]

(a) 0.5% methylcellulose (w/v) suspension.

(b) AUC(0-inf).

(c) AUC(0-24hr).

(d) 16 mg per person, assuming a body weight of 70 kg.

(e) Geometric least square mean value.

Table 3.b Pharmacokinetics of Ramelteon Across Species Following IV Administration

Species	Sex	Dose (mg/kg)	V _d (L/kg)	CL (mL/min/kg)	AUC(0-inf) (ng·hr/mL)	T _{1/2} (hr)	Report Number [Reference]
Rat	M	1	1.164	49.8	336.6	0.77	M-11-00781 [75]
Monkey	M	0.3	2.086	31	169.3	0.82	M-11-00782 [76]
Human	M	-0.02 (a)	2.04 (a)	13.0 (a)	18.2	1.80	TAK375/EC003 [77]

(a) assuming a body weight of 70 kg.

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2.6.4.4 Distribution

Dedicated *in vivo* studies of TAK-375 distribution were performed exclusively in the rat while *in vitro* studies utilized matrices from rat, monkey and human as well as the Caco-2 cell monolayer system. Distribution information was supplemented by toxicokinetic studies conducted in the rat, dog and monkey.

In vitro studies using rat, monkey and human blood demonstrated TAK-375 has low to moderate protein binding [M-11-00049.001A] between concentrations of 0.01 µg/mL and 1 µg/mL in all species, 75.0 – 83.3%. Blood partitioning indicated TAK-375 does not preferentially partition into red blood cells and in fact TAK-375 was found in slightly higher concentrations in plasma than in whole blood in rats [M-11-00781], a finding which was confirmed in human studies as well.

Tissue distribution of [¹⁴C]TAK-375 (1 mg/3.52 MBq/9.56 mL/kg) was assessed in male and female Sprague-Dawley rats [M-11-00747] using direct measurement of radioactivity as well as whole-body autoradiography. Sacrifice/sampling times of 15 min, 30 min, 2, 4, 8, 24, and 48 hr were included in this study. Results indicate that radioactivity was widely distributed

to tissues within 15 minutes after oral administration and highest amount of radioactivity observed within 30 minutes after oral administration of the test article. The highest concentrations of radioactivity observed in organs other than the GI tract were observed in the liver and kidney and whole-body radioactivity rapidly decreased following the 30 min time-point such that by 48 hr little radioactivity was observed.

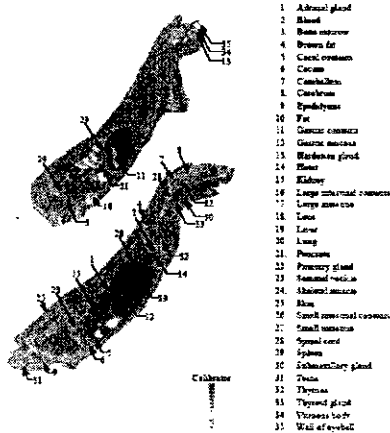


Fig. 2 Whole body autoradiograms at 30 min after a single oral administration of [¹⁴C]TAK-375 at a dose of 1 mg/kg to a male rat

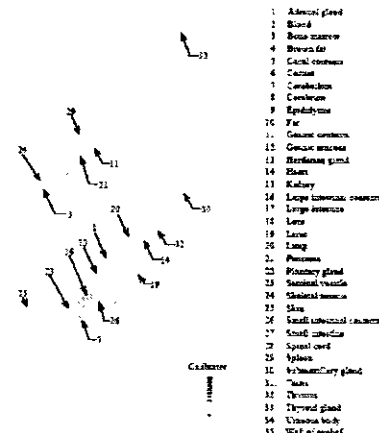


Fig. 3 Whole body autoradiograms at 48 h after a single oral administration of [¹⁴C]TAK-375 at a dose of 1 mg/kg to a male rat

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No gender differences in tissue distribution were noted. Another study [M-11-00-49.001A] which used [¹⁴C]TAK-375 to look primarily at absorption quantitatively assessed the radioactivity distribution to tissues of male rats by direct method at similar time-points and confirmed that C_{max} in each tissue was most frequently reached at a 30 min time-point. A comparison of distribution between tissues is reproduced in the Sponsor's table below:

Appears This Way
On Original

Table 1 Concentrations of ¹⁴C in tissues after oral administration of [¹⁴C]TAK-375 to rats

Tissue	Concentration of ¹⁴ C (μg TAK-375 equivalent/g)					
	Time after dosing (h)					
	0.5	2	4	8	24	48
Blood ¹⁾	0.290 ± 0.066	0.141 ± 0.025	0.066 ± 0.010	0.030 ± 0.003	0.010 ± 0.002	0.006 ± 0.001
Plasma ¹⁾	0.307 ± 0.069	0.152 ± 0.027	0.073 ± 0.012	0.033 ± 0.004	0.010 ± 0.002	0.005 ± 0.000
Brain	0.074 ± 0.024	0.027 ± 0.005	0.011 ± 0.002	0.005 ± 0.001	0.001 ± 0.000	0.001 ± 0.000
Spinal cord	0.088 ± 0.030	0.032 ± 0.005	0.013 ± 0.002	0.006 ± 0.001	0.002 ± 0.000	0.002 ± 0.001
Pituitary gland	0.280 ± 0.068	0.139 ± 0.025	0.056 ± 0.008	0.028 ± 0.003	0.010 ± 0.001	0.004 ± 0.000
Eye ball	0.077 ± 0.020	0.050 ± 0.008	0.023 ± 0.002	0.011 ± 0.002	0.003 ± 0.001	0.001 ± 0.000
Harder's gland	0.223 ± 0.064	0.150 ± 0.026	0.106 ± 0.020	0.077 ± 0.007	0.048 ± 0.011	0.020 ± 0.003
Submaxillary gland	0.271 ± 0.065	0.137 ± 0.028	0.066 ± 0.009	0.033 ± 0.007	0.006 ± 0.001	0.004 ± 0.000
Thyroid	0.271 ± 0.083	0.132 ± 0.025	0.075 ± 0.011	0.049 ± 0.013	0.022 ± 0.005	0.013 ± 0.002
Thymus	0.192 ± 0.043	0.096 ± 0.022	0.039 ± 0.005	0.019 ± 0.003	0.006 ± 0.000	0.004 ± 0.000
Heart	0.253 ± 0.067	0.113 ± 0.023	0.045 ± 0.007	0.020 ± 0.004	0.004 ± 0.001	0.003 ± 0.000
Lung	0.263 ± 0.064	0.124 ± 0.023	0.055 ± 0.006	0.028 ± 0.004	0.010 ± 0.002	0.007 ± 0.001
Liver	1.548 ± 0.307	0.745 ± 0.165	0.387 ± 0.063	0.170 ± 0.016	0.048 ± 0.003	0.031 ± 0.002
Spleen	0.246 ± 0.067	0.111 ± 0.022	0.047 ± 0.006	0.024 ± 0.004	0.007 ± 0.001	0.005 ± 0.000
Pancreas	0.267 ± 0.068	0.131 ± 0.024	0.067 ± 0.007	0.032 ± 0.005	0.006 ± 0.001	0.004 ± 0.001
Adrenal gland	0.377 ± 0.101	0.156 ± 0.028	0.063 ± 0.010	0.028 ± 0.006	0.009 ± 0.002	0.007 ± 0.001
Kidney	0.993 ± 0.193	0.563 ± 0.151	0.287 ± 0.107	0.106 ± 0.020	0.022 ± 0.004	0.014 ± 0.001
Testis	0.104 ± 0.029	0.078 ± 0.009	0.036 ± 0.004	0.015 ± 0.003	0.003 ± 0.001	0.002 ± 0.000
Skeletal muscle	0.180 ± 0.047	0.091 ± 0.015	0.037 ± 0.005	0.015 ± 0.003	0.003 ± 0.001	0.002 ± 0.000
Skin	0.174 ± 0.033	0.087 ± 0.013	0.040 ± 0.006	0.021 ± 0.003	0.010 ± 0.002	0.008 ± 0.000
Epididymal fat	0.064 ± 0.019	0.037 ± 0.011	0.011 ± 0.002	0.005 ± 0.002	0.003 ± 0.001	0.003 ± 0.000
Thigh bone	0.055 ± 0.011	0.044 ± 0.014	0.020 ± 0.004	0.013 ± 0.005	0.005 ± 0.000	0.004 ± 0.000
Bone marrow	0.207 ± 0.049	0.102 ± 0.019	0.045 ± 0.005	0.026 ± 0.003	0.008 ± 0.001	0.005 ± 0.001
Stomach	2.907 ± 0.562	1.268 ± 0.917	0.357 ± 0.228	0.049 ± 0.009	0.014 ± 0.003	0.007 ± 0.001
Intestine	0.673 ± 0.140	0.749 ± 0.220	0.759 ± 0.215	0.364 ± 0.137	0.045 ± 0.005	0.013 ± 0.004

Dose; 1mg/kg. Mean values ± S.D. (n=5).

1) μg/mL.

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The data obtained confirms the autoradiography results and reveals the tissues with highest concentrations (after the GI tract and blood/plasma) at the time of maximal exposure are the liver, kidney, adrenal gland and pituitary. Radioactivity is detected in the brain but is approximately 4-fold lower in concentration when compared to the amount detected in blood or plasma. By 48 hr, the concentration of radioactivity remaining in tissues, while significantly lower, was highest in liver, Harderian gland, kidney and intestine. Overall, the data does not suggest extensive absorption or sequestration in any specific tissue. Utilizing the data generated by this study, the half-life for radioactivity in these tissues was investigated in a separate study [M-11-00847] which concluded that for all tissues the $t^{1/2}_a \approx 1.2 - 3.3$ hr while the $t^{1/2}_b < 24$ hr except in the epididymal fat where this was apparently nearly 60 hr. The extent of [¹⁴C]TAK-375 distribution to the eyeball was examined in both albino and pigmented rats in order to determine if TAK-375 demonstrates affinity for melanin [M-11-00641]. There was only marginally greater radioactivity detected in pigmented rat eyeball when compared to the albino eyeball indicating TAK-375 and TAK-375 metabolites have only slight affinity for melanin. Repeated oral administration of [¹⁴C]TAK-375 to rats demonstrated evidence that tissue steady state was nearly achieved in 35 days with a slow and gradual reduction in tissue concentrations after administration was stopped [M-11-0049.001A].

As the metabolism of TAK-375 is extensive in nonclinical species (discussed in the next section) as well as humans, the Sponsor investigated the concentrations of TAK-375 and the active metabolite M-II in the brain and plasma of the rat [M-11-00783]. Male Sprague-Dawley rats were given a single oral administration of TAK-375 [M-11-00783] and blood was collected at 15 min, 30 min, 1, 2, 4, 6, and 8 hr after administration. Concentrations of TAK-375 and M-II were determined by β methods. Both TAK-375 and M-II

reached their peak concentrations in plasma at 15 min. Brain distribution was confirmed and peaked at 15 min for both TAK-375 and M-II with a rapid reduction in concentration over 8 hr. Brain concentrations reflected the prevailing plasma concentrations, being quite similar for both TAK-375 and M-II.

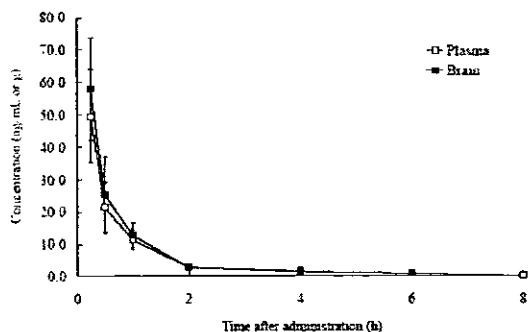


Fig 1 Concentration of TAK-375 in plasma and brain after a single oral administration of TAK-375 at a dose of 1 mg/kg to male rats

Each point with a vertical line represents the mean $\pm$ S.D. for three animals.

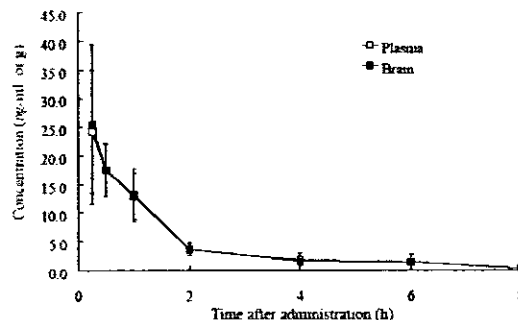


Fig 2 Concentration of M-II in plasma and brain after a single oral administration of TAK-375 at a dose of 1 mg/kg to male rats

Each point with a vertical line represents the mean $\pm$ S.D. for three animals.

In the rat, the M-II concentrations represented slightly less than half the circulating concentration of the parent TAK-375. A subsequent study was done assessing the concentrations of M-II and M-IV in the plasma and brain of the rat after oral administration with an additional analysis to determine the relative concentration of M-II that exists in plasma and brain of the rat in the diastereoisomer configuration (2*S*, 8*S*). Both M-II and M-IV were observed to circulate in plasma at similar concentrations. In contrast, M-II achieved brain concentrations as described previously which were similar to plasma while M-IV had relatively poor CNS penetration. Diastereomeric analysis indicated the great majority of the M-II metabolite circulating in plasma and distributed in the brain is of the (2*S*, 8*S*) configuration. The results of these studies suggest that both TAK-375 and the active metabolite M-II (2*S*, 8*S*) are capable of penetrating the blood brain barrier and exerting effects in the CNS.

The ability of TAK-375 to cross the placenta and enter breast milk was examined in two studies [M-11-00643 and M-11-00644, respectively] in the rat. [¹⁴C]TAK-375 was administered by oral gavage at a dose of 1 mg/kg to rats at Day 19 of pregnancy. Maternal rats were sacrificed at 30 min, 2, 4 or 24 hr after drug administration and radioactivity was assessed in the maternal plasma, fetal plasma, fetal tissue, placenta, and amniotic fluid. Consistent with the rapid distribution of TAK-375 exhibited in previously described studies, radiolabel concentration in each compartment analyzed was highest at 30 minutes after [¹⁴C]TAK-375 administration. Fetal plasma was shown to have the highest concentration of all compartments studied, exceeding by nearly 2-fold the maternal plasma concentration for up to 2 hr after administration. Within 4 hr of administration, the fetal plasma concentration curve resembled the maternal plasma concentration curve and little radioactivity (1-3% of max radioactivity concentration) was still present at 24 hr in maternal plasma, fetal plasma or fetal tissues. The placenta, fetal tissue and amniotic fluid overall exhibited equivalent or less radioactivity than the prevailing maternal plasma concentrations at all time-points. A subsequent study [M-11-00755] demonstrated the major circulating species in the fetal rat are TAK-375 and the M-II metabolite, consistent with the placental crossing of TAK-375 and its

conversion to M-II in the fetus. In the latter study 1 mg/kg [¹⁴C]TAK-375 was administered by oral gavage to lactating rats (Day 14 after parturition) and the concentration of radioactivity determined from maternal plasma, milk and mammary gland at 0.5, 2, 4, and 24 hr after drug administration. Maximal concentration of radioactivity was achieved at 0.5 hr post-dose in all compartments indicating the rapid distribution of TAK-375 or related metabolites into breast milk. Analysis of the relative concentrations of radioactivity between compartments revealed maternal plasma and mammary gland tissue concentrations were equivalent throughout the 24 hr observation while a 2.5 - 4 - fold higher concentration was obtained from milk when compared with maternal plasma and mammary gland tissue up to 4 hr after drug administration. By 24 hr post-dose all tissues were generally equivalent with very little radioactivity remaining.

2.6.4.5 Metabolism

TAK-375 metabolism was studied in a pharmacokinetic study in male Sprague-Dawley rats and male cynomolgus monkeys with oral and intraduodenal administration of [¹⁴C]TAK-375 as well as in an *in vitro* metabolic system to examine metabolism by specific cytochrome P450 enzymes [M-11-00049.001A, M-11-50]. The identification of [¹⁴C]TAK-375 and metabolites in rat and monkey plasma and urine utilized [

] Metabolites of TAK-375 characterized included 7 structures which were designated M-I, M-II, M-III, M-IV, M-IV, M-VI and an M-VII-glucuronide with M-VI and M-VII estimated. Several other unidentified metabolites were suggested but could not be identified in these studies.

Characterization of human metabolites identified several other metabolites not previously identified in the animal metabolism studies. The potential presence of these human metabolites in the nonclinical species used for toxicity testing was subsequently investigated in male Sprague-Dawley rat and cynomolgus monkey plasma using [] and [] methods after an oral administration of [¹⁴C]TAK-375 as well as using an incubation mixture of TAK-375 and M-II with human hepatic microsomes [M-11-752]. The results of this study identified another metabolite, designated M-IX, which is believed to be formed from hydroxylation. Furthermore, M-VII was positively identified and shown to be conjugated to glucuronic acid to form the M-VII glucuronide.

M-I	Oxidative cleavage
M-II	Hydroxylation
M-II	Oxidation of M-VII
M-IV	Hydroxylation of M-III or oxidation of M-II
M-V	Hydroxylation
M-VI	Hydroxylation of M-II or oxidation of M-V
M-VII	Exists as a glucuronide conjugate
M-IX	Hydroxylation

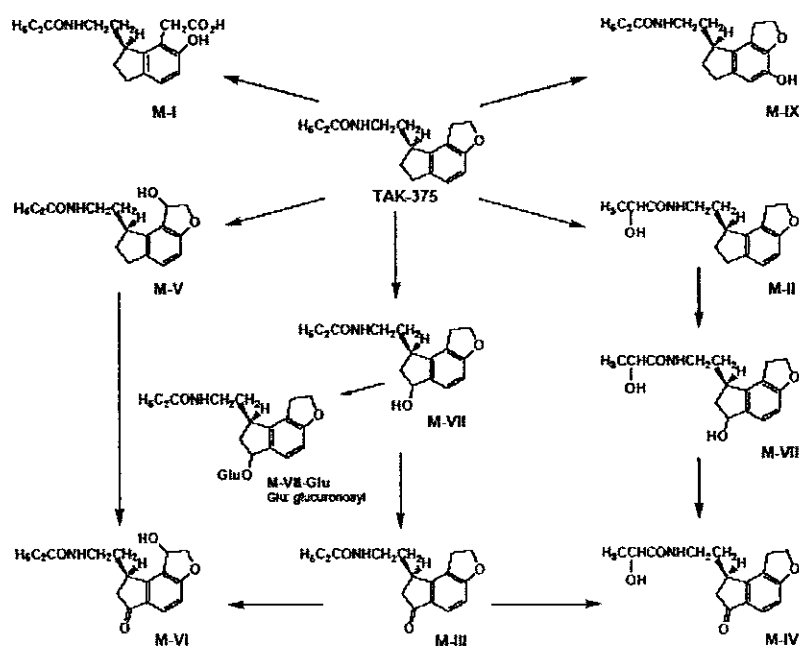


Figure 14 Postulated metabolic pathways of TAK-375

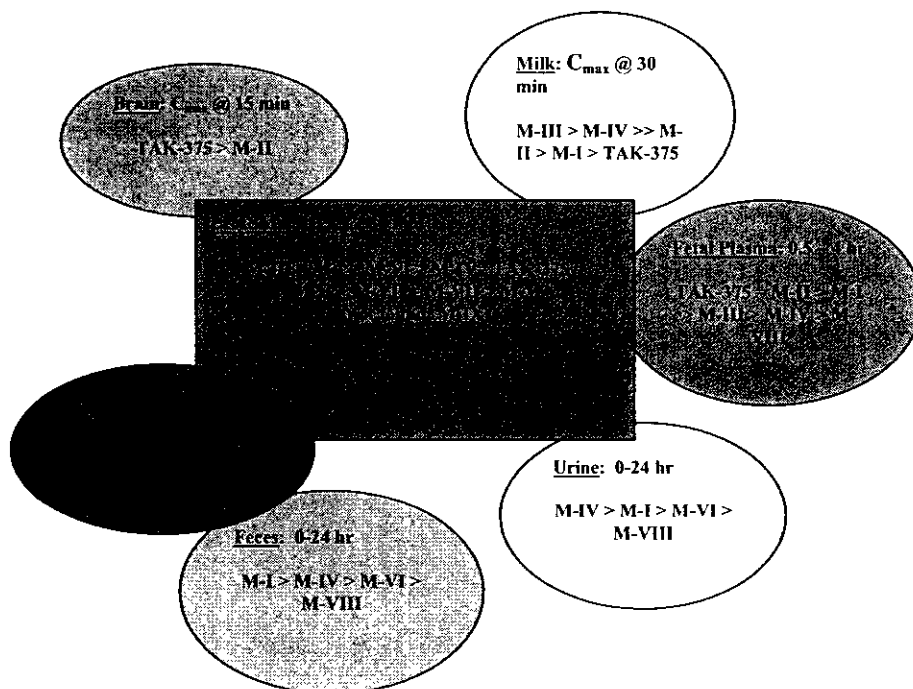
The significance of intestinal metabolism was examined in rats using instillation of 1 mg/kg [¹⁴C]TAK-375 into the jejunal loop of the intestines followed by sampling of portal blood between 0.5 – 2 hr post-administration [M-11-753]. Results of this study indicated that little if any metabolism occurs with the absorption process into portal circulation with unchanged TAK-375 representing 91-96% of radioactivity detected in portal blood within 2 hr of dosing.

A series of studies were completed in the male Sprague-Dawley rat in which the metabolites of TAK-375 were identified in the plasma, various excretory compartments and other locations of importance including maternal milk and fetal blood [M-11-00049.001A, M-11-00784, M-11-00785, M-11-00786, M-11-00754, M-11-00755]. In all studies, [¹⁴C]TAK-375 at a dose of 1 mg/kg was administered through oral, intravenous or intraduodenal routes and samples from the various regions or compartments analyzed by []

Metabolites detected in these studies are represented in the Figure below. The more significant observations from these studies in the rat include the differences in the major metabolites found in each matrix: Plasma of the rat contained more M-III than other metabolites while the bile and feces exhibited higher M-I concentrations and urine contained a higher percentage of M-IV. Assessment of brain and plasma concentrations after oral administration of [¹⁴C]TAK-375 showed rapid attainment of C_{max} at 15 minutes with the only species detected in the brain at this early time-point being the unchanged TAK-375 and the active metabolite M-II. When [¹⁴C]TAK-375 was given by oral gavage to pregnant rats on Day 19 of gestation the primary radioactive species observed in fetal plasma between 0.5 – 4 hr was the unchanged TAK-375 parent and M-II in similar concentrations followed by several other metabolites as described in the figure below at lower concentrations. Of interest, at 0.5 and 2 hr after oral administration to pregnant rats, the fetal blood contained significantly higher concentrations of TAK-375 and M-II than maternal blood (0.5 hr: 4-5-

fold; 2 hr: 11-13-fold) though by 4 hr these levels had dropped to more closely approximate maternal blood levels. When [¹⁴C]TAK-375 was administered to maternal rats on Day 14 of lactation and samples of milk were obtained and analyzed, the radioactive species with the highest concentrations at the time of C_{max}(radioactivity) were M-III and M-IV and the levels of these metabolites in the milk of lactating rats was 3-fold higher than that found circulating in plasma at this time-point. Furthermore, M-IV was retained at significantly higher levels than plasma for up to 4 hr after oral exposure to parent. Overall, however, the species observed were similar to that in maternal plasma.

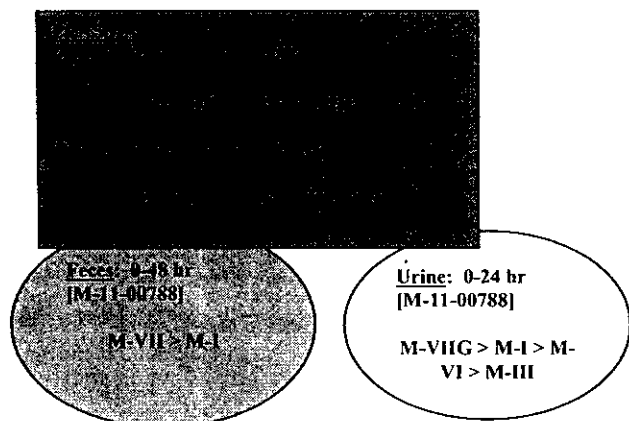
Identification of TAK-375 and Metabolites in the Rat (described in text)



A similar, though less extensive, set of studies were completed in the male cynomolgus monkey [M-11-00787, M-11-00788] to determine the major metabolites of interest in this species and characterize the levels of the parent and metabolites in various compartments. Male cynomolgus monkeys were administered [¹⁴C]TAK-375 at a concentration of 0.3 mg/kg and resulting radioactivity observed in the plasma were analyzed by γ

] The primary species observed were M-I, M-III, M-VII and M-VIIG (glucuronide conjugate) with the M-VIIG metabolite being in the greatest abundance. No unchanged TAK-375 or other metabolites were detected. Samples of urine from these animals collected for 24 or 48 hr revealed six metabolites: M-I, M-III, M-VI, M-VII, M-VIIG and M-VIII. Unchanged TAK-375 and other metabolites were not detected. Consistent with the data from plasma of monkeys, the species in highest abundance in the urine is M-VIIG over the first 24 hr of collection. Fecal samples revealed two metabolites M-I and M-VII. Unchanged TAK-375 and other metabolites were not observed.

Identification of TAK-375 and Metabolites in the Monkey



Notably the active metabolite M-II, which is major circulating species in the human, was not detected to any extent in the plasma, urine or feces of the cynomolgus monkey when TAK-375 was administered at this dose. In a 39-week repeat-dose toxicity and toxicokinetic study conducted in the cynomolgus monkey utilizing doses up to 200 mg/kg/day [M-11-232] M-II was not detected at a dose of 3 mg/kg/day but was observable with dosing $\geq$ 12 mg/kg/day. At the highest dose tested, the ratio of TAK-375:M-II exposure in plasma was 218:1.

The following table submitted by the Sponsor details the plasma exposure to TAK-375 and metabolites in rat and monkeys administered 1 mg/kg [^{14} C]TAK-375 [M-11-00049.001A]:

Table 3 Composition of ^{14} C in plasma of rats and monkeys given a single po dose of [^{14} C]TAK-375

Compound	AUC ($\mu\text{g}\cdot\text{h}/\text{mL}$) ¹⁾	
	Rat	Monkey
Total ^{14} C	0.931 $\pm$ 0.009 (100)	2.205 $\pm$ 0.098 (100)
TAK-375	0.034 $\pm$ 0.006 (3.7)	0.283 $\pm$ 0.067 (12.8)
M-I	0.092 $\pm$ 0.015 (9.9)	0.105 $\pm$ 0.032 (4.8)
M-II	0.044 $\pm$ 0.005 (4.7)	---
M-III	0.125 $\pm$ 0.018 (13.4)	---
M-IV	0.090 $\pm$ 0.017 (9.7)	---
M-V	0.065 $\pm$ 0.008 (7.0)	---
M-VI	0.107 $\pm$ 0.016 (11.5)	---
M-VII-G	---	0.785 $\pm$ 0.077 (35.6)
Others	0.375 $\pm$ 0.025 (40.1)	1.033 $\pm$ 0.121 (46.8)

Dose: 1mg/kg. Mean values $\pm$ S.D. (rat; n=5, monkey; n=4).

Figures in parentheses denote % of total ^{14} C.

1) TAK-375 equivalent (rat; AUC₀₋₄₈, monkey; AUC₀₋₂₄).

---: Not detected.

The following table submitted by the Sponsor provides a species comparison of the presence of metabolites of TAK-375:

Table 3.c Comparison of Identified Metabolites Across Species

Compound	Human	Mouse (a)	Rat	Rabbit (a)	Monkey
Ramelteon	P, U, F	P	P, U, F, B	P	P, F
M-I	P, U	P	P, U, F, B	P	P, U, F
M-II	P, U, F	P	P, U, F, B	P	P
M-III	P, U	P	P, U, F, B	P	P, U
M-IV	P, U	P	P, U, F, B	P	P
M-V	-	-	P, U, F, B	-	-
M-VI	-	-	P, U, F, B	-	U
M-VII	-	-	P	-	P, U, F
M-VII-Glucuronide	-	-	-	-	P, U, F
M-VIII	U, F	-	P, U, F	-	U
M-VIII-Glucuronide	P, U, F	-	-	-	-
M-IX-Glucuronide	P, U	-	B	-	-
Report Number [Reference]	TAK-375; EC004[81]; M-11-00327[93]	M-11-00560.002A [94]	M-11-49.001A[84]; M-11-00784[87]; M-11-00785[88]; M-11-00786[89]	M-11-188[95]	M-11-49.001A[84]; M-11-232[90]; M-11-00787[91]; M-11-00788[92]

P indicates plasma (serum used for human metabolite analysis), U, urine; F, feces; B, bile.
(a) Only plasma samples were analyzed.

The CYP isoforms involved in TAK-375 metabolism was examined as part of a profile of TAK-375 pharmacokinetics in animals [M-11-00049.001A] though this involved incubation of TAK-375 with human B-lymphoblastoid cells transfected with CYP cDNA. Nevertheless, CYP3A4, 1A1, 1A2, 2C9, and 2D6 were identified as being involved in metabolism of TAK-375. Further assessment using correlation between the metabolic activity of TAK-375 with data of metabolic activity using a specific substrate for each isoforms indicated that the principal CYP isoforms involved were 1A2, 3A4 and 2C9 as shown in Sponsor's table below:

Table 9 Correlation between metabolic rate of TAK-375 and CYP isoform specific activity

		CYP1A2	CYP2C9	CYP3A4
Elimination rate of TAK-375	Coefficient of correlation		0.650 <i>p</i> <0.05	0.800 <i>p</i> <0.01
Formation rate of M-II	Coefficient of correlation	0.918 <i>p</i> <0.01	-	-
Formation rate of M-III	Coefficient of correlation	-	0.635 <i>p</i> <0.05	0.976 <i>p</i> <0.01
Formation rate of M-V	Coefficient of correlation		0.595 <i>p</i> <0.05	0.989 <i>p</i> <0.01

-: no significant correlation was observed.

From this data it appears the initial metabolic steps involved in TAK-375 metabolism, as well as formation of the M-III and M-V metabolites involve CYP2C9 and 3A4 while formation of the active metabolite M-II is principally the action of CYP1A2.

2.6.4.6 Excretion

The routes of excretion and metabolite composition in excreta were explored by the Sponsor in single dose oral and intravenous studies of [¹⁴C]TAK-375 in the rat [M-11-00789, M-11-00049.001A] and in the cynomolgus monkey [M-11-00779, M-11-00049.001A].

Male Sprague-Dawley rats were administered 1 mg/kg [¹⁴C]TAK-375 in 0.5 w/v% methylcellulose, prepared just prior to administration, and the urinary, fecal and expiration excretion routes were analyzed for evidence of the radiolabel. Urine and feces were collected over dry ice chilled conditions while expired air was collected in a CO₂ absorbent solution. All matrices were assessed through direct measurement of radioactivity. Urine was sampled at 4, 8 and 24 hr after drug administration and thereafter at 24 hr intervals up to 168 hr post-dose. Feces were collected at 24 hr intervals up to 168 hr post-dose. Expired air was collected at 4, 8 and 24 hr and thereafter at 24 hr intervals up to 96 hr post-dose. Nearly half the radioactivity associated with the [¹⁴C]TAK-375 administered was recovered in the urine of rats within 8 hr (49.4 ± 2.7%) while within 24 hr 58.1% and 28.6% were recovered in the urine and feces, respectively. During this period little of the radioactivity was excreted through the respiratory route, accounting for 4.5% of total radioactivity. Therefore, more than 91% of radioactivity was recovered within the first 24 hr. At 72 hr following radiolabeled TAK-375 administration recovery was effectively complete with the urinary, fecal and expiratory routes accounting for 59.1%, 31.4%, and 4.8% of the total radiolabel, respectively, totaling 95.2% of administered radiolabel. At 168 hr after oral administration the total recovery was only increased a further 0.3% to 95.5%. Recovery percentages of radiolabel in urine, feces and expired air after intravenous administration of [¹⁴C]-TAK-375 was nearly identical to that seen with oral administration: 58.6%, 32.0% and 5.5% at 72 hr at which time it was effectively complete with a total recovery of 96.1%, gaining only 0.7% over the remaining 96 hr to total 96.8% recovery. Therefore, excretion patterns were highly similar between routes in the rat and the predominant method of elimination of TAK-375 and metabolites appears to be through urine though the fecal route plays a significant supporting role.

The excretion patterns associated with TAK-375 and its metabolites following a single oral or intravenous administration of 0.3 mg/kg [¹⁴C]TAK-375 were also investigated in the cynomolgus monkey [M-11-00779]. As in the rat, the urinary route proved the most important route of excretion with 72.2% of the radiolabel excreted into the urine and 4.8% excreted into the feces within 24 hr of oral administration while after the intravenous administration of [¹⁴C]TAK-375 approximately 74.3% of radiolabel was excreted in the urine and 5.6% in the feces within 24 hr. By 168 hr after oral and intravenous administration, the excretion of radioactivity into the urine was 77.2 and 78.2%, respectively, while in feces it was 11.3% and 12.3% of the total radioactivity administered. Recovery of radioactivity from urine and feces at 168 hr was 88.5% after oral administration and 99.5% of the total radioactivity after intravenous administration.

Evidence for TAK-375 excretion into bile was suggested by previous absorption and mass balance studies in the rat. As a result the Sponsor investigated the biliary excretion and

evidence for enterohepatic recirculation in a study in rats [M-11-00790]. Male Sprague-Dawley rats were bile-duct cannulated and were administered a single dose of 1 mg/kg [^{14}C]TAK-375. Feces, bile and urine were collected from rats and, consistent with the previously described studies, the urine was the major route of excretion (56%) but the bile accounted for 36% within the first 24 hr. A small fraction of radioactivity was found in the feces or remained in the GI system. Clearly the radioactivity associated with the fecal route in the previous study [M-11-00789] was a result of biliary excretion of TAK-375 and/or metabolites into the intestinal tract. When bile was pooled from an animal receiving [^{14}C]TAK-375 at 1 mg/kg and administered intra-duodenally to a separate rat, 21.1% and 19.2% of the administered radioactivity was excreted in the bile or urine while 55.6% was found in the feces which implies a significant degree of enterohepatic circulation may be in play as nearly 40% of the radioactivity placed into the intestinal tract was thought to have been reabsorbed. The results of this study demonstrate that 36% of the TAK-375 dose in the rat is excreted as unchanged or as a metabolite in the bile within 24 hr of oral administration and, furthermore, that TAK-375 and/or its metabolites are subject to significant enterohepatic circulation in this species.

Analysis of the TAK-375 species (i.e. parent and metabolites) contained within the excreta after oral administration was conducted in both rat and monkey [M-11-00049.001A] and indicated that very little of the unchanged TAK-375 is found in urine, bile or feces of nonclinical animals as documented the Sponsor's table below:

Table 11 Composition of radioactive materials in urine, feces, and bile of rats and monkeys given a single po dose of [^{14}C]TAK-375

Compound	Composition of ^{14}C (% of dose)				
	Rat			Monkey	
	Urine	Feces	Bile ¹⁾	Urine	Feces
Total ^{14}C	60.3 ± 4.2	29.5 ± 3.1	32.9 ± 3.8	78.0 ± 5.3	4.5 ± 2.7
TAK-375	0.1 ± 0.0	0.3 ± 0.1	0.8 ± 0.2	---	0.2 ± 0.2
M-I	7.2 ± 0.3	5.2 ± 1.1	2.6 ± 0.7	6.0 ± 2.6	---
M-II	0.4 ± 0.2	0.7 ± 0.2	1.2 ± 0.5	---	---
M-III	5.7 ± 0.6	2.4 ± 0.2	6.0 ± 1.1	---	---
M-IV	7.6 ± 1.3	1.8 ± 0.4	3.6 ± 0.4	---	---
M-V	1.3 ± 0.1	0.6 ± 0.2	1.3 ± 0.4	---	---
M-VI	11.9 ± 2.0	6.8 ± 1.9	1.8 ± 0.2	---	---
M-VII-G	---	---	---	29.3 ± 3.2	0.8 ± 0.6
Others	26.1 ± 1.5	11.8 ± 0.6	15.7 ± 1.9	42.8 ± 5.2	3.6 ± 1.9

Dose; 1mg/kg. Mean values ± S.D. (rat; n=5, monkey; n=4).

Data are for 0-24h samples.

1) Intraduodenal administration to biliary-cannulated rats.

---: Not detected.

Less than 1% of radioactivity detected in excreta of nonclinical species was revealed to be the TAK-375 parent and <1.2% of radioactivity was determined to be M-II indicating that the primary route of elimination of both TAK-375 and M-II is through hepatic metabolism.

2.6.4.7 Pharmacokinetic drug interactions

Drug interaction studies were conducted and submitted as part of the Clinical Pharmacology section of the NDA submission. Please see review by Dr. David Lee. A synopsis of the Sponsor's summary of these studies is reproduced verbatim below:

A study of the ability of ramelteon to inhibit the activity of the major human CYP isoforms indicated that ramelteon is unlikely to inhibit the metabolism of other compounds due to its high IC₅₀ values (>10 μ M) for inhibition of these CYP isoforms [99]. An extensive *in vitro* evaluation of the oxidative metabolism-based drug interaction potential of ramelteon was conducted, utilizing drugs that have been previously shown to be inhibitors of CYP1A2 [100,101], since this isoform has been identified as the major isoform responsible for the metabolism of ramelteon in humans. Results of these studies showed that the very potent CYP1A2 inhibitor, fluvoxamine, reduced the metabolism of ramelteon. Inhibition by other CYP1A2 inhibitors (such as ciprofloxacin, cimetidine, enoxacin, and mexiletine) was much weaker [102] and would be unlikely to significantly reduce the metabolism of ramelteon. Clinical studies confirmed that coadministration of potent CYP1A2 inhibitors significantly increased total plasma exposure of ramelteon [103]. Ramelteon exposure was also shown to increase in clinical studies, when coadministered with ketoconazole or fluconazole [104,105]. Drug-drug interactions mediated via P-glycoprotein are unlikely since ramelteon is neither a substrate for, nor an inhibitor of, this transporter [79,106]. Thus, ramelteon is unlikely to inhibit the metabolism of concomitant medications, whereas potent CYP1A2, CYP2C, or CYP3A4 inhibitors may inhibit ramelteon metabolism.

2.6.4.8 Other Pharmacokinetic Studies

TAK-375 was examined *in vivo* for an effect on hepatic drug metabolizing enzymes of male and female B6C3F₁ mice [M-11-00594] and Sprague-Dawley rats [M-11-00593].

Mice were administered TAK-375 by oral gavage at 0, 30, 100, 300 or 1000 mg/kg/day in 0.5 w/v methylcellulose for 1 week. These TAK-375 doses were identical to that used in the 2 year mouse carcinogenicity bioassay. The positive control used in this study was phenobarbital 100 mg/kg/day. Clinical signs such as decreased spontaneous activity and/or prone or lateral position was observed for the first two days of administration. Liver weights were increased in animals administered TAK-375 at $\geq$ 300 mg/kg/day and the phenobarbital group. The effects of TAK-375 on hepatic enzymes are documented in the Sponsor's table below:

Text Table 2. Summary of Hepatic Cytochrome Contents and Hepatic Drug-Metabolizing Enzymes

Sex	Enzymes									
	Males					Females				
	TAK-375		PB			TAK-375		PB		
Test compound	30		100			30		100		
Dose (mg/kg/day)	6		6			6		6		
No. of animals	6		6			6		6		
Cytochrome b ₅ contents	N	N	+25%*	+70%*	+78% ¹	N	+18%*	+39%*	+97%*	+78% ¹
Cytochrome P450 contents	N	N	+35%*	+87%*	+112% ¹	N	+23%*	+62%*	+121%*	+152% ¹
NADPH-cytochrome c reductase	N	+14%*	+24%*	+52%*	+61% ¹	N	+18%*	+30%*	+68%*	+62% ¹
Testosterone 2 α -hydroxylase	N	N	+32%*	+88%*	+80% ¹	N	+15%*	+45%*	+101%*	+89% ¹
Testosterone 6 β -hydroxylase	N	N	+103%*	+268%*	+199% ¹	N	N	+84%*	+250%*	+188% ¹
Testosterone 7 α -hydroxylase	N	N	N	+14%*	+23% ¹	N	N	N	+37%*	+27% ¹
Testosterone 16 α -hydroxylase	N	N	N	-21%*	+30% ¹	N	+26%*	+63%*	+124%*	+216% ¹
Testosterone 16 β -hydroxylase	N	N	N	N	+163% ¹	+22%*	+64%*	+102%*	+70%*	+342% ¹
Ethoxyresorufin O-deethylase	+29%*	+35%*	+23%*	+55%*	+181% ¹	N	+29%*	N	N	+74% ¹
p-Nitrophenol UDP-glucosyl transferase	N	N	+15%*	+42%*	+41% ¹	N	N	+19%*	+49%*	+56% ¹
Glutathione S-transferase	N	N	N	+34%*	N	N	N	+29%*	+114%*	+62% ¹

Values in the table indicate percentage against the control mean (+: increase, -: decrease).

N: No remarkable changes.

*: p<0.05, ¹: p<0.01 (significantly different from the control group)

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Examination of hepatic metabolizing enzymes revealed significant increases in enzyme content activity in both males and females in the 1000 mg/kg/day group while similar though less marked changes were noted in the 300 mg/kg/day group. Mild changes were seen in the 100 mg/kg/day group in both males and females while only a single enzyme was affected at 30 mg/kg/day. Enzymes most obviously affected by treatment in the mouse model were testosterone 6 β -hydroxylase, increased 268% in males and 250% in females. The most sensitive enzymes to exposure to TAK-375 were ethoxyresorufin O-deethylase which was increased in males and testosterone 16 β -hydroxylase in females observed at 30 mg/kg/day.

The companion study conducted in Sprague-Dawley rats [M-11-00593] utilized oral administration of 0, 15, 60, 250 and 1000 mg/kg/day in 0.5 w/v% methylcellulose for 1 week. These doses were identical to those used in the rat 2-year carcinogenicity bioassay. The positive control group used in this study was phenobarbital at 60 mg/kg/day. Mortality was observed in 3/10 females at 1000 mg/kg/day, all other animals survived treatment. Females that died demonstrated staggering, oligopnea and decreased spontaneous movement and deaths were considered treatment-related. Body weight was decreased in the 1000 mg/kg/day males only while liver weight was increased in males and females $\geq$ 250 mg/kg/day TAK-375 and in the positive control group. The effects of TAK-375 on hepatic metabolizing enzymes of the rat are shown in the Sponsor's table below:

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Text Table 2. Summary of Hepatic Cytochrome Contents and Hepatic Drug-Metabolizing Enzymes

Sex	Males					Females				
	TAK-375		PB			TAK-375		PB		
Test compound	15	60	250	1000	60	15	60	250	1000	60
Dose (mg/kg/day)	6	6	6	6	6	6	6	6	6	6
No. of animals										
Cytochrome b ₅ contents	N	N	+56%*	+77%*	+50% ¹	N	N	+41%*	+79%*	+32%*
Cytochrome P450 contents	N	N	+36%*	+81%*	+123% ¹	N	N	+27%*	+60%*	+73% ¹
NADPH-cytochrome c reductase	N	N	+81%*	+166%*	+87% ¹	N	N	+40%*	+124%*	+27% ¹
Testosterone 2 α -hydroxylase	N	N	N	-52%*	-52% ¹	N	N	+35%*	+20%*	+37% ¹
Testosterone 6 β -hydroxylase	N	N	+138%*	+228%*	+212% ¹	N	+93%*	+564%*	+732%*	+227% ¹
Testosterone 7 α -hydroxylase	N	+39%*	+53%*	+62%*	+132% ¹	N	N	+29%*	+55%*	+123% ¹
Testosterone 16 α -hydroxylase	N	N	N	+61%*	+48%*	N	N	+135%*	+960%*	+1193% ¹
Testosterone 16 β -hydroxylase	+22%*	+54%*	+417%*	+1777%*	+1707% ¹	N	N	N	+668%*	+819% ¹
Ethoxycarbazepine O-deethylase	+21%*	+56%*	+93%*	+113%*	+121% ¹	N	N	+38%*	+2%*	+35%*
p-Nitrophenol UDP-glucuronosyl transferase	N	N	+160%*	+438%*	+128% ¹	N	N	+146%*	+497%*	+81% ¹
Glutathione S-transferase	N	N	+47%*	+131%*	+68% ¹	N	+47%*	+105%*	+150%*	+85% ¹

Values in the table indicate percentage against the control mean (+: increase, -: decrease).

N: No remarkable changes.

*: p<0.05, ¹: p<0.01 (significantly different from the control group)

TAK-375 exposure produced marked increases in many hepatic drug metabolizing enzymes with dose levels of ≥ 250 mg/kg/day. Increased enzyme activity was observed with all dose levels (≥ 15 mg/kg/day) in males while females exhibited this change at dose levels ≥ 60 mg/kg/day. Testosterone 16 β -hydroxylase was the most highly affected enzyme in both males and females, $\uparrow 1777\%$ and $\uparrow 960\%$, respectively when compared with vehicle-treated animals. In general, TAK-375 exposure appeared to produce a very similar enzyme induction magnitude and pattern as compared with rats in the positive control group phenobarbital.

2.6.4.9 Discussion and Conclusions

TAK-375 appears to be well absorbed in all species tested, including humans, but also common to all species is a very high level of hepatic first pass metabolism rendering the bioavailability of TAK-375 to be quite low (highest in rat at 6.3%, lowest in monkey at 0.3%, with human having 1.8% bioavailability). Repeated oral administration of TAK-375 to rats in absorption studies demonstrated achievement of steady state plasma levels within 10 days while in toxicokinetic studies a gender effect was noted with females exhibiting higher plasma exposure to TAK-375 than males. This was not observed in other nonclinical species tested. Half life ($t^{1/2}$ α for the parent compound is short, < 1 hr in nonclinical species ($t^{1/2}$ β in rat is approximately 8 hr) and 1-2 hr in the human. Metabolites produced include 9 species, one of which, a hydroxylation product of the parent compound designated M-II, is active and demonstrates a pharmacologic profile similar to the parent compound though having about 1/10th the potency and efficacy of the parent based on *in vitro* pharmacology studies and *in vivo* behavioral (sleep induction) studies in nonclinical species. The M-II metabolite is the major circulating species in the human, 25-fold higher than TAK-375. M-II is observed in the mouse, rat and rabbit at levels far lower relative to TAK-375 but is still high enough to produce exposure multiples over the human exposure to the metabolite with the intended clinical therapeutic dose of Ramelteon. The monkey, however, has very little M-II metabolite as a circulating species likely due to even more rapid metabolism to subsequent metabolites. Toxicity studies with TAK-375 in this species would not produce human

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coverage for M-II with oral dosing. Consequently, the Sponsor utilized intravenous administration of the M-II metabolite to provide toxicologic qualification of this metabolite in this non-rodent model.

Hepatic metabolism of TAK-375 appears to largely involve CYP1A2, 2C9 and 3A4. Inhibition of these metabolizing enzymes with known inhibitors of these enzymes indicates interference with CYP1A2 can produce marked elevations in plasma TAK-375 exposure in clinical studies whereas interference with 2C9 and 3A4 produce a more modest effect. Repeated administration of TAK-375 *in vivo* produced a significant induction of hepatic drug metabolizing enzymes in rodents (both in toxicokinetic studies and in dedicated enzyme induction studies) and rabbits (the latter suggested by increased liver weight and reduction in plasma exposure with repeated dosing) but not in monkeys. TAK-375 and the major human circulating metabolite M-II do not appear to inhibit or induce CYP enzymes in *in vitro* studies with human hepatocytes or transfected lymphoblasts, nor does a reduction in exposure appear to occur with repeated dosing in human clinical studies.

TAK-375 and metabolites are well distributed throughout the body of the rat as determined by the spread of ^{14}C with highest concentrations outside of the GI tract occurring in the liver, kidney, adrenal gland and pituitary gland. Penetration of the blood-brain-barrier was demonstrated by radioactivity found in the brain of rats as well as subsequent identification of TAK-375 and M-II in the brain of rats within minutes of oral administration of TAK-375. No significant retention of radioactivity or accumulation was observed in distribution studies in the rat though a longer $t^{1/2}\beta$ was determined for radioactivity in the epididymal fat. TAK-375 crosses the placental barrier and enters breast milk therefore allowing fetal and neonatal exposure to TAK-375 and metabolites when the mother is exposed. Protein binding was moderate in all nonclinical species tested as well as in human plasma *in vitro*.

Excretion studies in nonclinical species indicate TAK-375 and metabolites are largely removed through the urinary route with a lesser involvement of the fecal route. In the rat, fecal excretion is almost entirely due to biliary excretion and is subject to enterohepatic recirculation. Analysis of the excreta of rat and monkey revealed less than 1% of unchanged TAK-375 was found in the urine, bile or feces of the rat or urine and feces of the monkey. This indicates that the elimination of TAK-375 is almost exclusively through hepatic metabolism in the nonclinical species used for toxicology studies.

2.6.4.10 Tables and figures to include comparative TK summary

Table 5 Fetal distribution of radioactivity after a single oral administration of ^{14}C TAK-375 at a dose of 1 mg/kg to pregnant rats.

Tissue	Time	Concentration (μg equiv/ml or μg)			
		0.5 h	2 h	4 h	24 h
Maternal plasma	0.447 ± 0.043	0.169 ± 0.024	0.093 ± 0.013	0.019 ± 0.002	
Fetal plasma	0.728 ± 0.111	0.243 ± 0.071	0.078 ± 0.007	0.006 ± 0.001	
Amniotic fluid	0.245 ± 0.035	0.142 ± 0.045	0.063 ± 0.007	0.011 ± 0.002	
Placenta	0.347 ± 0.068	0.156 ± 0.033	0.068 ± 0.010	0.010 ± 0.002	
Fetal tissue	0.420 ± 0.065	0.145 ± 0.024	0.058 ± 0.006	0.012 ± 0.002	

Each value represents the mean ± S.D. of three animals

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Table 5 Concentration of radioactivity in plasma, mammary gland and milk after a single oral administration of [¹⁴C]TAK-375 at a dose of 1 mg/kg to lactating rats

Tissues	Time	Concentration (µg equiv./mL or g)			
		0.5 h	2 h	4 h	24 h
Plasma		0.470 ± 0.021	0.103 ± 0.004	0.054 ± 0.012	0.007 ± 0.002
Mammary gland		0.490 ± 0.046	0.133 ± 0.012	0.070 ± 0.025	0.004 ± 0.001
Milk		1.132 ± 0.129	0.485 ± 0.044	0.261 ± 0.131	0.006 ± 0.002

Each value represents the mean ± S.D. of three animals.

METABOLISM FIGURES/TABLES

M-11-49001A (Pharmacokinetic study of TAK-375 in animals)

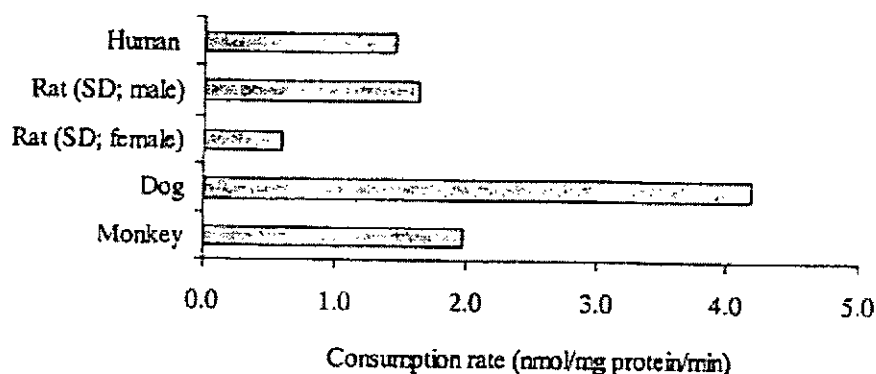


Fig. 10 *In vitro* metabolism of [¹⁴C]TAK-375 by hepatic microsomes of rats, dogs, monkeys, and humans

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EXCRETION FIGURES AND TABLES

Rat: Oral administration

Table 6-1 Cumulative excretion of radioactivity into urine, feces and expired air after a single oral administration of [¹⁴C]TAK-375 at a dose of 1 mg/kg to male rats

Time (h)	Cumulative excretion of radioactivity (% of dose)			Total
	Urine	Feces	Expired air*	
4	37.4 ± 0.4	-	3.5 ± 0.2	N.E.
8	49.4 ± 2.7	-	3.8 ± 0.3	N.E.
24	53.1 ± 0.7	26.6 ± 1.4	4.5 ± 0.1	91.2 ± 2.0
48	53.9 ± 0.5	31.2 ± 0.2	4.7 ± 0.1	94.8 ± 0.4
72	59.0 ± 0.5	31.4 ± 0.3	4.8 ± 0.1	95.2 ± 0.4
96	59.1 ± 0.5	31.5 ± 0.3	4.8 ± 0.1	95.4 ± 0.4
120	59.1 ± 0.5	31.6 ± 0.2	4.8 ± 0.1	95.5 ± 0.4
144	59.2 ± 0.5	31.6 ± 0.2	4.8 ± 0.1	95.5 ± 0.4
168	59.2 ± 0.5	31.6 ± 0.2	4.8 ± 0.1	95.5 ± 0.4

- Not collected
 N.E. Not estimated
 Each value was represented as the mean ± S.D. for three animals
 * Collection of expired air was stopped at 96 h.

Rat: Intravenous administration

Table 6-2 Cumulative excretion of radioactivity into urine, feces and expired air after a single intravenous administration of [¹⁴C]IAK-375 at a dose of 1 mg/kg to male rats

Time (h)	Cumulative excretion of radioactivity (% of dose)			
	Urine	Feces	Expired air*	Total
4	45.7 ± 0.7	-	3.5 ± 0.5	N.E.
8	49.6 ± 3.6	-	4.8 ± 0.5	N.E.
24	57.6 ± 1.1	29.1 ± 2.9	5.3 ± 0.6	92.0 ± 1.6
48	58.4 ± 1.2	31.7 ± 2.5	5.4 ± 0.6	95.6 ± 0.9
72	58.6 ± 1.2	32.0 ± 2.5	5.5 ± 0.6	96.1 ± 0.9
96	58.8 ± 1.2	32.2 ± 2.5	5.5 ± 0.6	96.5 ± 0.9
120	58.9 ± 1.2	32.3 ± 2.5	5.5 ± 0.6	96.7 ± 0.9
144	59.0 ± 1.2	32.3 ± 2.5	5.5 ± 0.6	96.8 ± 0.9
168	59.0 ± 1.2	32.3 ± 2.5	5.5 ± 0.6	96.8 ± 0.9

- Not collected.

N.E. Not estimated.

Each value was represented as the mean ± S.D. for three animals.

* Collection of expired air was stopped at 96 h.

Rat: Intraduodenal administration

Table 1-6 Recovery of radioactivity in bile, urine and feces after a single intraduodenal administration of [¹⁴C]IAK-375 at a dose of 1 mg/kg to male rats

Time (h)	Recovery of radioactivity (% of dose)			
	Bile	Urine	Feces*	Total
2	28.2 ± 4.0	-	-	N.E.
4	33.8 ± 4.0	48.1 ± 6.5	-	N.E.
8	35.4 ± 4.2	52.9 ± 4.5	-	N.E.
24	36.0 ± 4.1	56.0 ± 4.7	3.7 ± 0.8	95.6 ± 0.3

- Not collected.

N.E. Not estimated.

* The recovery in feces includes that in gastric and intestinal contents.

Excretion Across Species

Table 6.a Excretion of Total Radioactivity Across Species Following a Single Oral Dose of [¹⁴C]ramelteon

Species	Sex	Route	(mg/kg)	Percent of Dose Excreted (%)			Report Number
				Urine	Feces	Total	
Rat	M	IV	1	59.0	32.3	96.8(a)	M-11-00789
	M	Oral	1	59.2	31.6	95.5(a)	M-11-00789
Monkey	M	Oral	1	61.3	32.2	93.8(a)	M-11-49.001A
	M	IV	0.3	78.2	12.3	90.5	M-11-00779
	M	Oral	0.3	77.2	11.3	88.5	M-11-00779
Human	M	Oral	1	80.2	9.9	90.1	M-11-49.001A
	M	Oral	0.23(b)	84.3	4.0	88.3	EC004

(a) Including small amount radioactivity recovered in expired air.

(b) Each subject (assuming 70 kg body weight) received 16 mg [¹⁴C]ramelteon.

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Toxicokinetic analysis of TAK-375 and metabolites from M-11-176: 26-week oral toxicity study in the rat

Table 1 Toxicokinetic parameters for TAK-375, M-I, M-II, M-III and M-IV in rats

Dose (mg/kg/day)	Analyte	No. of Animals	Male (n=2)			Female (n=2)			Total (n=4)			
			Time (h)	Cmax (ng/mL)	AUC _{0-24h} (ng·h/mL)	Time (h)	Cmax (ng/mL)	AUC _{0-24h} (ng·h/mL)	Time (h)	Cmax (ng/mL)	AUC _{0-24h} (ng·h/mL)	
2.5	TAK-375	1a	0.1 (0.1)	27 (21)	27 (21)	0.1 (0.1)	40 (10)	40 (10)	0.1 (0.1)	40 (10)	33 (26)	
		96h	0.5 (0.5)	134 (24)	134 (24)	0.5 (0.5)	297 (110)	297 (110)	0.5 (0.5)	263 (144)	100 (40)	
		172h	0.3 (0.3)	191 (31)	191 (31)	0.3 (0.3)	211 (26)	211 (26)	0.3 (0.3)	221 (103)	214 (81)	
	M-I	172h	0.5 (0.5)	278 (18)	285 (57)	0.5 (0.5)	481 (143)	376 (41)	0.5 (0.5)	340 (137)	301 (43)	
		172h	0.2 (0.2)	167 (96)	132 (91)	0.2 (0.2)	217 (103)	346 (33)	0.2 (0.2)	217 (150)	216 (158)	
		172h	0.3 (0.1)	237 (94)	290 (137)	0.3 (0.1)	472 (151)	333 (111)	0.3 (0.1)	265 (146)	461 (156)	
	M-IV	172h	0.7 (0.3)	69 (34)	129 (97)	0.7 (0.3)	261 (27)	612 (117)	0.7 (0.3)	157 (105)	371 (288)	
		1a	0.3 (0.1)	471 (217)	346 (121)	0.3 (0.1)	1010 (497)	710 (174)	0.3 (0.1)	742 (400)	560 (240)	
		96h	0.3 (0.1)	1790 (321)	2007 (459)	0.3 (0.1)	1740 (382)	1977 (81)	0.3 (0.1)	2365 (706)	1992 (443)	
	10	TAK-375	172h	0.3 (0.1)	1197 (232)	1631 (392)	0.3 (0.1)	2101 (86)	2114 (476)	0.3 (0.1)	2242 (899)	1047 (506)
			1a	0.1 (0.1)	1519 (145)	2312 (118)	0.1 (0.1)	1654 (496)	1664 (418)	0.1 (0.1)	1337 (314)	1970 (419)
			96h	0.6 (0.4)	246 (14)	713 (117)	0.5 (0.1)	1172 (272)	1950 (481)	0.1 (0.2)	700 (518)	1235 (733)
M-III		172h	0.3 (0.1)	1121 (18)	2319 (710)	0.3 (0.1)	1916 (102)	2418 (117)	0.3 (0.1)	1537 (770)	2914 (1041)	
		172h	1.0 (0.1)	184 (43)	282 (206)	1.0 (0.1)	643 (224)	2383 (949)	1.0 (0.1)	416 (326)	1541 (1108)	
		1a	0.3 (0.1)	2017 (1010)	4783 (844)	0.3 (0.1)	4317 (1077)	9423 (2330)	0.3 (0.1)	6137 (1776)	7901 (3048)	
40		TAK-375	96h	0.5 (0.1)	4193 (1712)	11490 (3140)	0.5 (0.1)	7063 (2440)	13197 (2103)	0.5 (0.1)	6648 (2700)	12542 (3783)
			172h	0.6 (0.4)	2450 (332)	7329 (2099)	0.4 (0.1)	11610 (2693)	12437 (7214)	0.5 (0.3)	2043 (1143)	13382 (7976)
			172h	0.6 (0.4)	3033 (380)	8464 (1941)	0.3 (0.1)	2320 (473)	3116 (816)	0.3 (0.2)	3702 (311)	2054 (2040)
		M-I	172h	1.0 (0.1)	324 (152)	1213 (1612)	1.0 (0.1)	3712 (211)	9773 (809)	1.0 (0.1)	1881 (1506)	6620 (2100)
			172h	0.7 (0.3)	2810 (603)	9070 (1814)	1.0 (0.1)	2174 (1612)	26110 (2491)	0.8 (0.2)	3162 (1112)	17949 (10072)
			172h	1.0 (0.1)	272 (16)	1312 (411)	2.0 (1.2)	1310 (167)	7112 (1618)	1.7 (1.0)	716 (120)	4183 (3428)
	150	TAK-375	1a	0.4 (0.1)	9087 (1469)	47170 (10335)	0.3 (0.1)	21093 (10394)	93100 (14832)	0.4 (0.1)	15490 (1058)	70425 (28139)
			96h	0.7 (0.3)	7227 (4202)	20957 (16012)	0.3 (0.1)	15173 (1234)	43107 (14631)	0.4 (0.2)	11300 (4871)	14112 (11030)
			172h	0.3 (0.1)	2615 (1818)	26753 (11214)	0.2 (0.1)	13976 (4933)	17076 (17169)	0.6 (0.2)	10350 (4419)	43664 (21626)
		M-I	172h	0.1 (0.1)	7790 (1200)	22483 (7719)	0.4 (0.1)	1002 (49)	10564 (2007)	0.5 (0.1)	1800 (612)	14371 (817)
			172h	1.0 (0.1)	794 (274)	2472 (411)	0.4 (0.2)	1017 (676)	20008 (1908)	0.9 (0.2)	2151 (1094)	14151 (10132)
			172h	0.8 (0.2)	4710 (229)	26150 (1144)	0.8 (0.2)	8240 (2829)	22613 (10330)	0.9 (0.3)	7361 (3344)	49006 (26497)
M-IV		172h	1.0 (0.1)	263 (87)	3611 (402)	2.1 (1.3)	1114 (170)	10916 (2246)	1.7 (1.0)	739 (337)	4464 (311)	

Mean (SD).

Best Possible Copy

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.5.3 Pharmacokinetics: Absorption After a Single Dose

Test Article: TAK-375				
Report No.: M-11-00781 M-11-00748 M-11-00749 (rat) M-11-00642 (dog) M-11-00782 (monkey) TAK-375 EC004 (human) M-11-00750 (Caco-2 cell monolayers)				
Species:	Rat	Dog	Monkey	Human
Gender (M/F):No. of animals:	M:3	M:4	M:4	M:6
Feeding condition:	Fed	Fed	Fed	Fasted
Vehicle/formulation:	0.5% MC	0.5% MC	0.5% MC	Aqueous solution
Method of administration:	Gavage	Gavage	Gavage	Oral
Dose (mg/kg):	1	1	0.3	1.5 mg
Sample:	Plasma	Plasma	Plasma	Serum
Analyte:	TAK-375	TAK-375	TAK-375	TAK-375
Assay:	T			
PK Parameters:	J			
Tmax (hr)	0.25	0.1	0.33	0.314
Cmax (ng/mL)	17.5	112	0.525	25.0
AUC (ng·hr/mL)	21.3	129	0.414	25.7
(Time for calculation -hr)	(0-infinite)	(0-24)	(0-infinite)	(0-24)
tl_{1/2} (hr)	0.54 (alpha)	2.4 (beta)	0.71	1.24

Additional Information:

A single oral dose was well absorbed in all species, however, bioavailability was relatively low due to extensive first pass metabolism. [M-11-00781, M-11-00786, M-11-00782, M-11-00783] In rats, the major absorption sites were duodenum, jejunum and ileum, with stomach and colon as the minor absorption sites [M-11-00748], and most of the absorption was via portal vein [M-11-00749]. Rimegeleon is highly permeable and it is not a substrate for p-glycoprotein in a permeability study using Caco-2 cell monolayers [M-11-00750] 0.5% MC, 0.5% w/v methylcellulose solution

2.6.5.5A Pharmacokinetics: Organ Distribution, Single Dose

Test Article: TAK-375
 Report No.: M-11-00849.001A
 M-11-00847
 M-11-00842

Species	Rat
Gender (M/F) No. of animals:	M 5
Feeding condition:	Fed
Vehicle/Formulation:	0.5% MC
Method of administration:	Oral Gavage
Dose (mg/kg):	1
Radioisotope:	¹⁴ C
Specific activity:	4.0 MBq/mg
Sampling time:	0.5, 2, 4, 8, 24 and 48 hr

Tissues/Organ	Concentration (ng/g)					
	0.5 hr	2 hr	4 hr	8 hr	24 hr	48 hr
Blood	290	141	66	30	10	17.9
Plasma	307	152	73	33	10	15.2
Brain	74	27	11	5	1	18.7
Lung	263	124	55	28	10	20.9
Liver	1548	745	387	170	48	17.1
Kidney	993	563	287	106	22	14.4
Testis	194	78	36	15	3	14.5
Muscle	130	91	37	15	3	14.5

Additional Information:
 Spinal cord, pituitary gland, eye ball, Harder's gland, submaxillary gland, thyroid, thymus, heart, spleen, pancreas, adrenal gland, skin, epididymal fat, thigh bone, bone marrow, stomach and intestine were examined but not shown. TAK-375 and M-II were distributed into the brain [M-11-00783, M-11-00640].
 The C_{max} concentrations of radioactivity in eyeballs of pigmented rats were slightly higher compared to that in albino rats, but the decline of radioactivity in pigmented rats was very rapid [M-11-00641], suggesting that melanin-related material does not have high affinity towards melanin.
 0.5% MC: 0.5w/v, methylcellulose solution

2.6.5.9 Pharmacokinetics: Metabolism In Vivo

Gender (M/F) No. of animals:	Rat: M 3	Monkey: M 4	Human: M 6
Feeding condition:	Rat: Fed	Monkey: Fed	Human: Fast
Vehicle/Formulation:	Rat: 0.5% MC	Monkey: 0.5% MC	Human: Aqueous solution
Method of administration:	Rat: Gavage	Monkey: Gavage	Human: Drinking
Dose (mg/kg):	Rat: 1	Monkey: 0.3	Human: 0.23
Radioisotope:	Rat: ¹⁴ C	Monkey: ¹⁴ C	Human: ¹⁴ C
Specific activity:	Rat: 3.52 MBq/mg	Monkey: 3.52 MBq/mg	Human: 3.16 MBq/16 mg

Species	Sample	Sampling Time or Period	% of Dose in Sample	% of Compound in Sample											Report No.
				Parent	M-I	M-II	M-III	M-IV	M-V	M-VI	M-VII	M-VIII	M-IX	M-X	
Rat	Plasma	0.5 hr	-	5	8.3	5.8	9.9	6.3	3.3	6.9	4.7	-	4.9	-	M-11-00784
	Urine	0-24 hr	58.1	-	3.5	-	-	6.5	-	1.7	-	-	0.4	-	M-11-00785
	Bile (a)	0-24 hr	36.0	-	3.7	-	-	6.5	-	0.5	-	-	-	1.4	M-11-00786
	Feces	0-24 hr	28.6	-	3	-	-	1	-	0.3	-	-	0.1	-	M-11-00785
Monkey	Plasma	0.25 hr	-	-	5.1	-	0.2	-	-	-	5	24	-	-	M-11-00787
	Urine	0-24 hr	72.2	-	1.2	-	-	-	-	0.4	2.9	16	1	-	M-11-00788
	Feces	0-48 hr	3.3	-	0.3	-	-	-	-	-	0.6	-	-	-	M-11-00788
Human	Urine	0-3 hr	-	7.5	2.1	3.0	0.8	2.2	-	-	-	-	-	-	TAK-375 EC004
	Urine	0-48 hr	84.3	-	0.1	0.3	0.08	3.3	-	-	-	-	2.1	1.4	
	Feces	0-48 hr	4.0	-	-	-	-	-	-	-	-	-	-	-	

Additional Information:
 (a) Intraduodenal administration for collection of bile
 (b) M-VIII-Glucuronide was detected in human serum and urine but was not detected in either rats or monkeys
 0.5% MC: 0.5w/v, methylcellulose solution

2.6.5.10 Pharmacokinetics: Metabolism In Vitro

		Test Article: TAK-375
		Report No.: M-11-00650
In vitro metabolism of [¹⁴C]TAK-375 by baculovirus-infected-insect cell microsomes expressing human CYP isoforms		
Study System:	Baculovirus-infected-insect cell microsomes expressing human CYP isoforms	
Reaction Time:	60 min	
Initial Concentration:	50 µmol/L	

CYP isoforms	Consumption rate (pmol/hr/pmol P450) of TAK-375
CYP1A1	325.8
CYP1A2	468.3
CYP2A6	1.8
CYP2B6	48.6
CYP2C8	246.6
CYP2C9*1	12.8
CYP2C19	357.8
CYP2D6	19.6
CYP3E1	2.3
CYP3A4	362.3

Additional Information:
 In the CYP1A1, 1A2, 2C19 and 3A4, less than 30% of TAK-375 remained after reaction [M-11-00650]. A similar study was conducted with Lymphoblastoid cell microsomes expressing human CYP isoforms [M-11-00649.001A].

2.6.5.10 Pharmacokinetics: Metabolism In Vitro (continued)

		Test Article: TAK-375
		Report No.: M-11-00650
In vitro metabolism of [¹⁴C]TAK-375 by baculovirus-infected-insect cell microsomes expressing human CYP isoforms		
Study System:	Baculovirus-infected-insect cell microsomes expressing human CYP isoforms	
Reaction Time:	10 min	
Initial Concentration:	50 µmol/L	

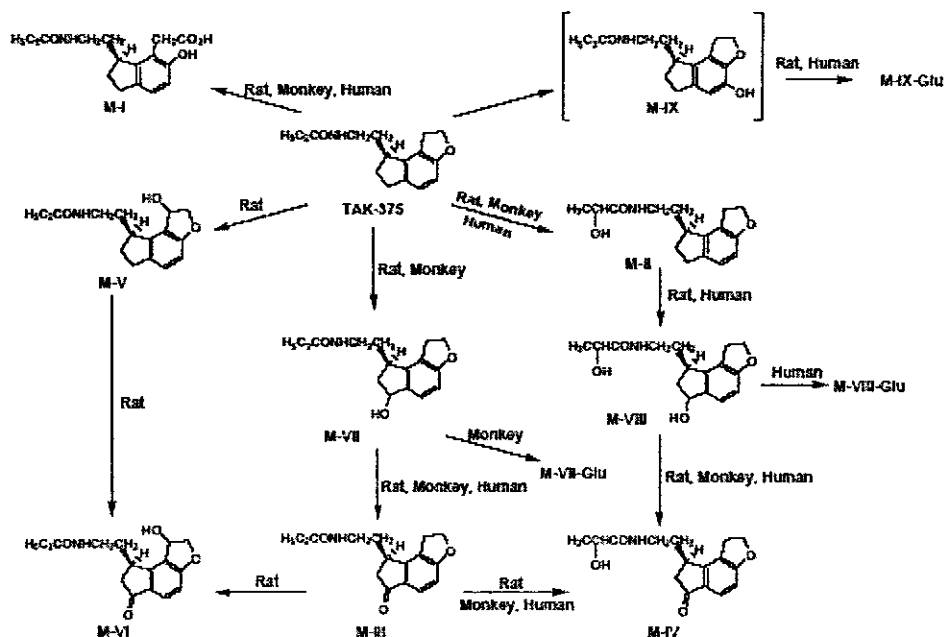
CYP isoforms	Consumption rate (pmol/hr/pmol P450) of TAK-375
CYP1A1	1388.0
CYP1A2	3180.0
CYP2C19	1239.6
CYP3A4	1341.6

Additional Information:
 The reactions were conducted with 50 pmol P450/mL and 10 min, because less than 30% of TAK-375 remained after the reactions with 100 pmol P450/mL and 60 min [M-11-00650].

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2.6.5.11 Pharmacokinetics: Possible Metabolic Pathways

(Illustrates possible metabolic map indicating species in which metabolic reactions occur.)



2.6.5.12 Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes

Test Article: TAK-375, M-II, Zolpidem
 Report No: M-11-00295

Effect of TAK-375 on marker enzyme activities in lymphoblastoid cell microsomes expressing human CYP isoforms:

Type of Study:	In vitro inhibition
Study System:	Lymphoblastoid cell microsomes expressing human CYP isoforms
Marker Activities:	CYP1A1, 1A2 CYP2A6 CYP2B6 CYP2C8, 2C9(Arg), 2C9(Cys) CYP2C19 CYP2D6 CYP2E1 CYP3A4
	7-ethoxyresorufin O-deethylation coumarin 7-hydroxylation ethoxycoumarin O-deethylation tolbutamide hydroxylation (S)-mephenytoin 4'-hydroxylation (+)-bufuralol 1'-hydroxylation 4-aminophenol hydroxylation testosterone 6beta-hydroxylation

IC50 ranges of TAK-375, M-II and Zolpidem for CYP isoforms

	TAK-375	M-II	Zolpidem
CYP1A1	100 µmol/L	>100 µmol/L	>100 µmol/L
CYP1A2	100 µmol/L	>100 µmol/L	100 µmol/L
CYP2A6	100 µmol/L	>100 µmol/L	100 µmol/L
CYP2B6	100 µmol/L	100 µmol/L	100 µmol/L
CYP2C8	10 - 100 µmol/L	100 µmol/L	100 µmol/L
CYP2C9(Arg)	100 µmol/L	100 µmol/L	100 µmol/L
CYP2C9(Cys)	100 µmol/L	100 µmol/L	100 µmol/L
CYP2C19	10 - 100 µmol/L	100 µmol/L	100 µmol/L
CYP2D6	100 µmol/L	100 µmol/L	100 µmol/L
CYP2E1	100 µmol/L	100 µmol/L	100 µmol/L
CYP3A4	10 - 100 µmol/L	100 µmol/L	10 - 100 µmol/L

Additional Information

In vitro inhibition of TAK-375 was also conducted in another study [M-11-000-9 001A], and similar results were observed. In vitro, TAK-75 showed some weak inhibition to the melatonin metabolism in rats [M-11-00649].

2.6.5.13 Pharmacokinetics: Excretion

		Test Article: TAK-375					
		Report No.: M-11-00789 (rat) M-11-00779 (monkey) M-11-00049 001A					
Species	Rat	Monkey					
Gender (M/F):No. of animals:	M/3	M/4					
Feeding condition:	Fed	Fed					
Vehicle/Formulation:	0.5% MC	0.5% MC					
Method of administration:	Oral	Oral					
Dose (mg/kg):	1	0.3					
Analyte:	¹⁴ C	¹⁴ C					
Assay:							
Excretion Route Time	Urine (%)	Feces (%)	Expired air (%) ^(a)	Total (%)	Urine (%)	Feces (%)	Total (%)
0-4 hr	37.4	-	3.5	-	49.8	-	-
0-8 hr	49.4	-	3.8	-	62.2	-	-
0-24 hr	58.1	28.6	4.5	91.2	72.2	4.8	77.0
0-48 hr	58.9	31.2	4.7	94.8	74.7	8.3	83.0
0-72 hr	59.0	31.4	4.3	95.2	75.9	9.7	85.6
0-96 hr	59.1	31.5	4.8	95.4	76.4	10.4	86.9
0-120 hr	59.1	31.5	4.8	95.5	76.8	10.9	87.7
0-144 hr	59.2	31.5	4.8	95.5	77.0	11.1	88.1
0-168 hr	59.2	31.6	4.8	95.5	77.2	11.3	88.5

Additional Information:
 Similar results were obtained in another study [M-11-00049 001A]. The total radioactivity recovery after a single IV dose to rats was 96.5% and about 5.5% was recovered in the expired air [M-11-00789]. The total radioactivity recovery after a single IV dose to monkeys was 90.5% [M-11-00779].
 0.5% MC: 0.5w/v%, methylcellulose solution
 (a) Collection of expired air was stopped at 96 h.

2.6.5.14 Pharmacokinetics: Excretion into Bile

		Test Article: TAK-375		
		Report No.: M-11-00790 M-11-00049 001A		
Species	Rat			
Gender (M/F):No. of animals:	M/4			
Feeding condition:	Fed			
Vehicle/Formulation:	0.5% MC			
Method of administration:	Intraduodenal			
Dose (mg/kg):	1			
Analyte:	¹⁴ C			
Assay:				
Excretion Route Time	Bile (%)	Urine (%)	Feces (%) (a)	Total (%)
0-4 hr	33.8	48.1		
0-8 hr	35.4	52.9		
0-24 hr	36.0	56.0	3.7	95.6

Additional Information:
 In a separate study in rats, the total radioactivity recovered in bile and urine in 24 hours after an intraduodenal administration of bile (0-24 hr) collected from other rats dosed with 1 mg/kg [¹⁴C]TAK-375 was 21.1 and 19.2%, respectively, suggesting that rimegefon-related radioactivity undergoes enterohepatic recirculation. Similar results were obtained in another study [M-11-00049 001A].
 0.5% MC: 0.5w/v%, methylcellulose solution
 (a) Recovery includes gastric and intestinal contents.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

General toxicology studies conducted by the Sponsor are summarized in the following table provided by the Sponsor:

2.6.7.1 Toxicology Overview

Type of Study	Species and Strain	Method of Admin.	Duration of Dosing	Daily Dose (mg/kg)	GLP Compliance (a)	Testing Facility	Study/Report Number
Single-dose toxicity	SD rats	Gavage 0.5% MC	Single dose	0, 300, 600, and 2000	Yes		2635/AC/M-11-16
Single-dose toxicity	SD rats	IV DMSO	Single dose	0, 6, 20, and 60	Yes		2785/AC/M-11-78
Single-dose toxicity	SD rats	IV 30% PEG	Single dose	0, 0.02, 0.2, and 2	Yes		01-443/AC/M-11-347
Single-dose toxicity: escalating dose	Cynomolgus monkeys	Gavage 0.5% MC	Escalating dose	0, 2, 20, 200, and 2000	Yes		2610/AC/M-11-11
Repeat-dose toxicity	SD rats	Gavage 0.5% MC	2 weeks	0, 30, 200, 600, and 2000	No		2625/rs/M-11-00610
Repeat-dose toxicity	SD rats	Gavage 0.5% MC	2 weeks	0, 30, 200, and 600	No		2662/rs/M-11-00611
Repeat-dose toxicity	SD rats	Gavage 0.5% MC	4 weeks	0, 10, 40, 150, and 600	Yes		2699/SUM-11-27
Repeat-dose toxicity	SD rats	Gavage 0.5% MC	4 weeks, 4 and 13 weeks-recovery	0, and 600	Yes		2784/SUM-11-110
Repeat-dose toxicity	SD rats	Gavage 0.5% MC	26 weeks	0, 2.5, 10, 40, and 150	Yes		2873/CH/M-11-176
Repeat-dose toxicity	Cynomolgus monkeys	Gavage 0.5% MC	2 weeks	0, 2, 20, 200, and 1000	No		2653/rs/M-11-00606
Repeat-dose toxicity	Cynomolgus monkeys	Gavage 0.5% MC	2 weeks	0, 2, 20, 200, and 1000	No		2683/rs/M-11-00607
Repeat-dose toxicity	Cynomolgus monkeys	Gavage 0.5% MC	4 weeks	0, 3, 12, 50, and 200	Yes		2700/SUM-11-26-1
Repeat-dose toxicity	Cynomolgus monkeys	Gavage 0.5% MC	39 weeks	0, 3, 12, 50, and 200	Yes		8833/M-11-232
Repeat-dose toxicity	Cynomolgus monkeys	IV 30% PEG	1 week	M-II (2.5, 8s) 0, 1, 3, and 10	No		3034/rs/M-11-156
Repeat-dose toxicity	Cynomolgus monkeys	IV 30% PEG	4 weeks	M-II (2.5, 8s) 0, 1, 3, and 10	Yes		SBL18-92/M-11-192

Type of Study	Species and Strain	Method of Admin.	Duration of Dosing	Daily Dose (mg/kg)	GLP Compliance (a)	Testing Facility	Study/Report Number
Genotoxicity-Reverse mutation assay	S. typhimurium E. coli	In vitro	-	11.2, 128, 320, 800, 2000 and 5000 µg/plate	Yes		3643 M-11-13
Genotoxicity-Mammalian cell gene mutation test	L5178Y Mouse lymphoma cells	In vitro	-	50, 100, 150, 200, 300, 400 and 500 µg/plate	Yes		G97CB86.702/M-11-19-1
Genotoxicity-Chromosomal aberration test	Chinese hamster lung cells	In vitro	-	56.3, 113, 225, 450 and 900 µg/plate	Yes		3644 M-11-17-1
Genotoxicity-Micronucleus test	mice	Gavage 0.5% MC	2 days	0, 250, 500, and 1000	Yes		2634 GE/M-11-20
Genotoxicity-Micronucleus test	SD rats	Gavage 0.5% MC	2 days	0, 300, 1000, and 2000	Yes		3643 M-11-18
Genotoxicity-Unscheduled DNA synthesis assay	SD rats	Gavage 0.5% MC	Single dose	0, 1000, and 2000	Yes		3841 M-11-22
CA Range-finding	B6C3F ₁ mice	Gavage 0.5% MC	4 weeks	0, 50, 150, 500, and 1500	No		2788 ca-M-11-00609
CA Range-finding	B6C3F ₁ mice	Gavage 0.5% MC	13 weeks	0, 30, 100, 300, and 1000	Yes		B-4203 M-11-178
CA Carcinogenicity	B6C3F ₁ mice	Gavage 0.5% MC	24 months	0, 30, 100, 300, and 1000	Yes		B-4204/M-11-00560.002A
CA Range-finding	Wistar rats	Gavage 0.5% MC	4 weeks	0, 50, 150, 600, and 1500	No		2790 ca-M-11-00603
CA Range-finding	Wistar rats	Gavage 0.5% MC	13 weeks	0, 40, 150, 600, and 1200	Yes		2934 CA/M-11-00171.001A
CA Carcinogenicity	SD rats	Gavage 0.5% MC	24 months	0, 15, 60, 250, and 1000	Yes		B-4416 M-11-00561.002A
CA Other studies	In vitro	-	-	-	No		M-11-00598
CA Other studies: Effects on drug-metabolizing hepatic enzymes	B6C3F ₁ mice	Gavage 0.5% MC	1 week	0, 30, 100, 300, and 1000	No		C-B082 M-11-00594
CA Other studies: Effects on melatonin, thyroid hormones, and Harderian glands	B6C3F ₁ mice	Gavage 0.5% MC	4 weeks	0, 3, 30, and 300	No		03-375 ca M-11-00709

Type of Study	Species and Strain	Method of Admin.	Duration of Dosing	Daily Dose (mg/kg)	GLP Compliance (a)	Testing Facility	Study/Report Number
CA Other studies: Effects on hepatic drug-metabolizing enzymes	SD rats	Gavage 0.5% MC	1 week	0, 15, 60, 250, and 1000	No		C-B081 M-11-00593
CA Other studies: Effects on plasma hormones	SD rats	Gavage 0.5% MC	1 week	0, 5, 20, 60, and 200	No		2787 ca M-11-73
CA Other studies: Effects on plasma estradiol and progesterone	SD rats	Gavage 0.5% MC	About 5 weeks	0, 15, 60, and 250	No		02-362 fa M-11-00565
CA Other studies: Effects on plasma melatonin and DHEA	SD rats	Gavage 0.5% MC	4 weeks	0, 15, 60, 250, and 1000	No		02-351 ca M-11-00569
CA Other studies: Effects on testosterone and luteinizing hormone	SD rats	Gavage 0.5% MC	1 week	0, 250, and 1000	No		03-489 fa M-11-00659
CA Other studies: Effects on testosterone and luteinizing hormone	SD rats	Gavage 0.5% MC	1 week	0, 250, and 1000	No		33-699 fa M-11-00690
CA Other studies: Effects on testosterone and luteinizing hormone	SD rats	Gavage 0.5% MC	4 weeks	0, 250, and 1000	No		04-056 fa M-11-00691
Reproductive and developmental toxicity FEE	SD rats	Gavage 0.5% MC	M (♂) F (♀)	0, 5, 70 (F), 50 (F) M, and 600 (F)	Yes		2701 FE M-11-37
Reproductive and developmental toxicity FEE	SD rats	Gavage 0.5% MC	Single dose Toxicokinetic	0, 50, and 600	Yes		2742 FE M-11-38
Reproductive and developmental toxicity FEE	SD rats	Gavage 0.5% MC	M (♂) F (♀)	0, 20 (F) F, 60 (F) M, and 200 (F)	Yes		2782 FE M-11-34
Reproductive and developmental toxicity range-finding EFD	SD rats	Gavage 0.5% MC	F-G6-G17	0, 10, 100 and 1000	No		2630 ca M-11-20.001
Reproductive and developmental toxicity EFD	SD rats	Gavage 0.5% MC	F-G6-G17	0, 10, 40, 150 and 600	Yes		2701 FE M-11-36

Single-dose toxicology studies

Single dose toxicology studies were completed in both Sprague-Dawley rat and cynomolgus monkey.

In the rat, oral administration of TAK-375 produced mortality at 2000 mg/kg in a single female with no death in males. Death in the female was preceded by clinical signs of decreased respiration and lying on one side. Clinical signs at 2000 mg/kg p.o. in both males and females consisted of decreased motor activity, prone position, decreased touch-escape response and ataxia. Intravenous administration of TAK-375 in rats up to 60 mg/kg produced a similar constellation of clinical signs which exhibited a dose-related increase in severity but was potentially confounded by use of DMSO as the vehicle which demonstrated similar though more mild signs. Hypothermia was observed at 60 mg/kg and appeared to be related to TAK-375. Lower doses of TAK-375, up to 2 mg/kg i.v., and use of a different vehicle indicated only a mild reduction in body weight gain in males and females ($\downarrow 6\%$ ♂, $\downarrow 13\%$ ♀) at the high dose at the end of a 14-day observation period. This effect on body weight gain appeared due a carry-over effect from the first day.

In the monkey, TAK-375 was administered by oral gavage and demonstrated vomiting with doses ≥ 200 mg/kg and salivation and pale oral mucosa at 2000 mg/kg. Mild decrease in body temperature (up to 1.7°C) and decrease in heart rate was noted with all doses ≥ 20 mg/kg which is considered to be a pharmacologic extension of this melatonin receptor agonist. All effects recovered with 14-day follow-up observation though several animals receiving 2000 mg/kg exhibited reduced body temperature for 8 days following this acute treatment.

Repeat-dose toxicology studies

Major repeat-dose toxicology studies were conducted in the B6C3F₁ mouse, the Sprague-Dawley and Wistar rat, and cynomolgus monkey. The principal species used for general repeat-dose toxicology studies were the rat and the cynomolgus monkey. The mouse was used in several dose range-finding studies to support the dosing strategy for the 2-year carcinogenicity bioassay in this species. Comparison across species of the major toxicities found in these repeat-dose toxicology studies are discussed below.

TAK-375 produced evidence of CNS toxicity in all species when given at a relatively high dose. In the mouse, decreased spontaneous locomotor activity was noted at a dose level ≥ 300 mg/kg/day while oligopnea was observed in males at and above this dose though in females it was seen only at 1000 mg/kg/day. Mice also exhibited prone or lateral position at a dose level ≥ 300 mg/kg/day in males and at 1000 mg/kg/day in females. These responses fully resolved within several days of treatment. In the rat, decreased spontaneous locomotor activity was also noted at doses ≥ 600 mg/kg/day in both Sprague-Dawley and Wistar strains. Salivation was observed with doses ≥ 40 mg/kg/day but this was considered to be due to the bitterness of the test article and therefore not a manifestation of toxicity. Administration of TAK-375 to the monkey produced a reduction in spontaneous locomotion when measured 1 hr post administration of dose levels of ≥ 12 mg/kg/day and which were observed throughout the 39-week study. An increased incidence and severity of emesis was also commonly observed in monkey at was noted with dosing ≥ 50 mg/kg/day. Convulsions were seen in 2 male monkeys at the 200 mg/kg/day dose level on a single occasion and were preceded by

observations of decreased muscle tone and oligopnea. These animals did not exhibit convulsive behavior subsequently.

Hematologic changes that were notable or common between species included an increase in platelets observed across species. In mice this was observed to occur with dosing ≥ 1000 mg/kg/day while in rat an increase was noted with TAK-375 doses > 150 mg/kg/day in females in the chronic study and at ≥ 600 mg/kg/day in both sexes in a shorter 13-week study. Although no statistically significant changes in platelets were found when analyzed by group in monkeys, 2/4 high dose (200 mg/kg/day) males had platelet counts that were above the historical data range. Female cynomolgus monkeys did not show this effect at the highest dose studied. Mild reductions ($<5\%$) in red blood cell parameters (RBC, hemoglobin and hematocrit) were noted in rats in the chronic study at the maximum dose level of 150 mg/kg/day and were indicative of a mild anemia with consequent histopathologic finding of splenic cell proliferation and increased spleen weight. This observation was not noted in either mouse or monkey animal models. In the chronic 39-week study of TAK-375 in the monkey, a dose-related but mild decrease in APTT was significant at the 200 mg/kg/day level. Although dismissed by the Sponsor, 4/8 animals at this dose level were reported to have values below the historical control range.

Increased serum transaminases AST and ALT were noted in the mouse with dosing ≥ 1000 mg/kg/day though these increases were generally less than 2-fold. Total cholesterol was found to be significantly lower than controls with dose levels 300 – 1000 mg/kg/day in the 13-week study but was increased at 1500 mg/kg/day in the 4 week toxicity study. A general reduction in the A/G ratio was noted in all studies at 1000 mg/kg/day and was perhaps related to decreased plasma albumin levels in males at 1000 mg/kg/day and females at ≥ 300 mg/kg/day.

In the rat, total cholesterol was increased with doses ≥ 150 mg/kg/day in females and 600 mg/kg/day in males and the elevation was less than 2-fold. A mildly decreased A/G ratio was noted in females only at the highest dose used in the chronic 26-week study (150 mg/kg/day). Total protein was increased, however, in both males and females at a dose ≥ 150 mg/kg/day with males having an increase in albumin levels. Plasma calcium was also noted to be increased in both males and females relative to control with a dose ≥ 150 mg/kg/day.

Repeat-dose toxicity studies of 4 weeks or 39-weeks duration in the monkey revealed several changes in clinical chemistry worth of note. Elevated ALT was observed with dosing at 200 mg/kg/day in both studies though in the chronic 39-week study this was more marked in males and became more severe over the course of the study. ALT was increased more than 4-fold in males when compared both to control animals and their own baseline values. Females at 200 mg/kg/day appeared to have a trend towards elevated ALT ($\uparrow 75\%$ vs. controls) but this was not statistically significant and the sample size was small ($n=4/\text{sex}/\text{group}$). Triglycerides were elevated in males at 200 mg/kg/day but not in females.

Clear TAK-375 target organs were identified in all nonclinical species upon gross necropsy and histopathologic assessment of tissues and were relatively consistent:

Mouse

In mice, the liver was observed to dark in females, visibly enlarged and exhibiting an increase in weight with doses ≥ 300 mg/kg/day. Microscopic assessment revealed hepatocellular hypertrophy in the centrilobular region which was rated as slight to mild in severity. Biochemical analysis of tissue from necropsy samples indicated a significant increase in the activity of hepatic drug metabolizing enzyme aminopyrine N-demethylase relative to controls but no effect was noted on aniline hydroxylase. Other organs with findings included the adrenal gland of both males and females in which vacuolization of cortical cells were noted at 1000 mg/kg/day. Vacuoles were observed in the zona fasciculata and in both sexes were considered fine and slight in nature though the adrenal gland in females also demonstrated evidence of hypertrophy. The ovary also appeared to be a target of TAK-375 in mice and demonstrated evidence of vacuolization of interstitial cells with a dose of 1500 mg/kg/day for 4-weeks but this was not observed at lower doses or in a 13-week study with maximum dose of 1000 mg/kg/day.

Rat

Rats also demonstrated TAK-375 alterations in liver morphology and histology. Liver weight was increased significantly in rats at dose levels ≥ 150 mg/kg/day and centrilobular hepatocellular hypertrophy was evident with dosing ≥ 40 mg/kg/day in the chronic 26-week toxicity study. Analysis of hepatic enzyme activity from the 26-week study did not reveal a significant increase at 150 mg/kg/day though some reduction in plasma exposure to TAK-375 was noted in the study. Statistically significant increases in hepatic enzymes were noted, however, in 4-week toxicity studies a significant increase in aminopyrine N-demethylase activity, aniline hydroxylase was observed with dosing ≥ 150 mg/kg/day. Thyroid weight was increased with TAK-375 dosing of 4-weeks or longer with dosing ≥ 40 mg/kg/day in males and 150 mg/kg/day in females. The increase in weight was paralleled by findings of follicular cell hypertrophy. Assessment of thyroid hormones T4 and T3 as well as TSH indicated a reduction in T3 and increase in circulating TSH with doses ≥ 150 mg/kg/day. T4 was reduced only at a dose of 1500 mg/kg/day though an increase in UDP-glucuronosyltransferase enzyme activity for T4 was noted in a 4-week rat toxicity study at a dose of 600 mg/kg/day. As in mice, the adrenal gland of rats increased in weight with exposure to TAK-375 and demonstrated a diffuse cortical vacuolization observed in females at dose levels ≥ 40 mg/kg/day and in males at 150 mg/kg/day. Also replicating the changes observed in mice, the ovaries of rats administered TAK-375 at doses ≥ 40 mg/kg/day exhibited vacuolization of interstitial cells. Other target organs included the kidney with increased weight without underlying histopathology; the spleen which was increased in weight in the 26-week study and demonstrated evidence of extramedullary hematopoiesis, considered related to the mild anemia displayed in this study.

Monkey

The primary target organ in monkeys administered TAK-375 up to 200 mg/kg/day in 4-week and 39-week toxicity studies was the liver. Liver weight was increased in the 4-week study at doses ≥ 200 mg/kg/day without evidence of an underlying histopathology though increased ALT was noted in males and females at 200 mg/kg/day. Samples of the liver were analyzed and demonstrated increased aminopyrine N-demethylase activity in a minority of animals with doses ≥ 12 mg/kg/day and in aniline hydroxylase activity with doses ≥ 50

mg/kg/day. In the 39-week study liver weight was increased at 200 mg/kg/day and under microscopic assessment of this tissue revealed diffuse hepatocellular hypertrophy that was not zonal in nature. Further indication that the increase in liver weight was not an adaptive response to enzyme induction were the observations that no enzyme induction was detected in contrast to the 4-week study and that ALT increases were marked in the males (> 4-fold vs. controls) and elevated though this did not reach statistical significance in females.

Genetic toxicology:

TAK-375 and by extension its metabolites were tested in a Bacterial Reverse Mutation (Ames) assay, Mammalian cell gene mutation test, and a Chromosomal aberration assay *in vitro*. *In vivo* studies conducted included both a micronucleus test in mice as well as rats. Additionally, an *in vivo/in vitro* Unscheduled DNA Synthesis study was conducted in the rat hepatocyte model for further examination of the genetic toxicity of TAK-375 and its metabolites.

TAK-375 was tested in *S. typhimurium* strains TA100, 98, 1535, 1537 and *E. Coli* WP2uvrA to detect reverse mutation of the histidine or tryptophan gene. Positive controls produced the expected increase in revertant colonies capable of growing in histidine or tryptophan-deficient media. Revertant colonies were not increased in any tested strain with TAK-375 incubation at concentrations of 51.2 – 5000 µg/plate in either the S9 metabolic activation condition or without S9 metabolic activation. Subsequent analysis of metabolites produced in the +S9 condition demonstrated concentrations of M-I – M-IV such that these metabolites could be considered to be adequately tested in this study.

TAK-375 was tested in mouse lymphoma LY5178Y/TK^{+/+} cells at concentrations of 50 – 300 µg/mL in the +S9 metabolic activation condition and 100 – 500 µg/mL in the –S9 condition, with appropriate doses determined from a dose range-finding study which demonstrated excessive cytotoxicity as measured by inhibition of suspension growth at 500 µg/mL. The positive control produced an increase in mutant frequency over the observed saline control and colony sizing of resulting mutants indicated primarily small colonies. Incubation of LY5178Y/TK^{+/+} cells with TAK-375 under either +S9 or –S9 conditions produced 80-90% mitotic inhibition at the highest concentrations tested and no increase in mutant frequency was observed at any concentration in the presence or absence of S9 metabolic activation. Subsequent analysis of metabolites produced in the +S9 condition demonstrated concentrations of M-I – M-IV such that these metabolites could be considered to be adequately tested in this study.

The clastogenic potential of TAK-375 was assessed in a chromosomal aberration assay using Chinese hamster lung (CHL) cells. Concentrations used in the definitive study were 56.3 – 450 µg/mL for both 24 and 48 hr tests. Concentrations used in the –S9 condition were identical but in the +S9 condition concentrations were 113 – 900 µg/mL. Visible precipitation was observed at a concentration of 337 µg/mL both in the presence of and absence of S9. Increased chromosomal aberrations were observed at concentrations ≥ 113 µg/mL in the direct method, and 450 µg/mL in the +S9 metabolic activation condition. Definite reduction in mitotic index and cell survival rates were observed at doses at which increased chromosomal aberrations were seen. A lack of clastogenic effect in the –S9 condition was considered of questionable validity by the reviewing pharmacologist/toxicologist due to the use of an improper positive control

(cyclophosphamide) which is appropriate only for the +S9 condition, and which did not produce chromosomal aberrations. Nevertheless, TAK-375 was considered to produce clastogenicity as studied in the CHL cell system.

Rats (Sprague-Dawley) and mice (C57BL/6J) were used in separate studies designed to examine the ability of TAK-375 to produce micronucleated polychromatic erythrocytes. In the rat study, TAK-375 doses used were 500, 1000 or 2000 mg/kg/day administered by oral gavage while in the mouse study doses used were 250, 500 or 1000 mg/kg. In both studies TAK-375 was administered at the doses described once daily for 2 days with examination of femoral bone marrow erythrocytes 24 hr after the last administration of drug. In both studies, TAK-375 administration did not result in an increase in micronucleated polychromatic erythrocytes at any dose level. TAK-375 was therefore considered negative in the *in vivo* micronucleus study in both mice and rats.

An investigation into the ability of TAK-375 to cause direct DNA damage was evaluated using the *in vivo/in vitro* Unscheduled DNA Synthesis assay in rat hepatocytes. Rats were administered a single oral dose of 1000 or 2000 mg/kg and DNA synthesis in isolated hepatocytes was evaluated at 2 or 16 hr after drug administration. No evidence of increased net nuclear grains, indicative of DNA damage, was observed at either dose but was found with the positive control used to validate the assay. Therefore TAK-375 was considered negative in the UDS assay.

Carcinogenicity¹:

The tumorigenic potential of TAK-375 was assessed in B6C3F₁ mice as well as Sprague-Dawley rats in 2 year bioassays. TAK-375 doses used in the mouse study were 0, 30, 100, 300 or 1000 mg/kg/day by oral gavage. Dosage levels in the rat study were 0, 15, 60, 250 or 1000 mg/kg/day by oral gavage. Plasma concentrations of TAK-375 and metabolites M-I through M-IV were assessed on Day 1, Week 13 and Week 52.

In the mouse study, excessive mortality was observed at the 1000 mg/kg/day level which necessitated the early termination of this group at Week 94. The presumptive cause of death at this dose was the development of hepatic tumors. In surviving animals hepatic tumors were found in males and females with doses of ≥ 300 mg/kg/day and consisted of hepatic adenoma, carcinoma and blastoma in males and in females adenoma was demonstrated with TAK-375 dose levels of ≥ 300 mg/kg/day while carcinoma was observed only in the 1000 mg/kg/day female group. Combining adenoma and carcinoma in males reduced the level at which a significant difference in tumors was found relative to control group animals to 100 mg/kg/day. The NOAEL dose in male mice was therefore 30 mg/kg/day and was associated with a plasma concentration which provides a 103-fold safety margin for TAK-375 when compared with the expected human clinical exposure while for the active metabolite M-II the safety margin is 3-fold over the human exposure and the margin for M-IV is 64-fold. The NOAEL dose for tumors in female mice was 100 mg/kg/day which provides a safety margin of 827-fold for TAK-375 and 12-fold for M-II over the expected human therapeutic exposure while M-IV has a 105-fold margin. Evidence for hepatic enzyme induction was suggested in both species with marked reductions in plasma exposure with dose levels of ≥ 100 mg/kg/day in both males and females.

¹ Note also that the M-IV metabolite is included due to the demonstration that exposure to this metabolite is expected to exceed the threshold for qualification of circulating metabolites based on the latest information from the Sponsor.

Administration of TAK-375 to rats for up to 104 weeks at dose levels of 0, 15, 60, 250 or 1000 mg/kg/day was associated with excessive mortality in the 1000 mg/kg/day group females which required termination of this group during Week 94. Early mortality with dose levels $\leq$ 250 mg/kg/day were ascribed to the development of pituitary tumor while at 1000 mg/kg/day this was considered to be due to TAK-375-induced hepatic tumors or intoxication, the latter explanation given for deaths early in the study period and occurring shortly after administration of the test article. TAK-375-induced hepatic tumors were found in males with dose levels $\geq$ 250 mg/kg/day (adenoma at $\geq$ 250 mg/kg/day, carcinoma at 1000 mg/kg/day) and in females with dose levels $\geq$ 60 mg/kg/day (adenoma $\geq$ 60 mg/kg/day, carcinoma at 1000 mg/kg/day). Administration of TAK-375 at 1000 mg/kg/day was also associated with the development of Leydig cell tumors in males. The rat liver tumor NOAEL was considered to be 60 mg/kg/day in males and 15 mg/kg/day in females and corresponded to plasma levels of TAK-375, M-II, and M-IV which in males provided a safety margin of 1,419-fold, 12-fold and 27-fold, respectively, over the expected human therapeutic exposure at 8 mg/day Ramelteon, while in females the safety margin is considered to be 472-fold, 16-fold and 70-fold for TAK-375, M-II, and M-IV metabolites, respectively. Evidence for hepatic enzyme induction was noted for males and females in groups receiving $\geq$ 250 mg/kg/day TAK-375 based on the reduction in exposure to TAK-375 observed over the duration of the study.

Reproductive toxicology:

The effects of TAK-375 on reproductive potential as well as embryofetal development and pre- and postnatal development was studied in the Sprague-Dawley rat and Japanese White rabbit (the latter in the embryofetal study only).

Administration of TAK-375 by oral gavage at dose levels of 0, 6, 60, or 600 mg/kg/day to males 4 weeks prior to mating through mating and in females from 2 weeks prior to mating through Day 6 of pregnancy. Unexpected mortality in females occurred at the 600 mg/kg/day dose level within days of the start of TAK-375 treatment with death in 9/20 of these animals. This was unexpected as a previous 4-week toxicology study in rats with dosing at this level was not associated with mortality. Assessment of surviving females in this group and the other TAK-375-treated groups indicated the following reproductive effects: females at a dose level $\geq$ 60 mg/kg/day demonstrated irregularity in the estrus cycle, reductions in the number of implants and reduction in the number of live embryos. Pre- and post-implantation loss was observed to be increased in the 60 mg/kg/day treatment group only and thus was considered to be incidental while a decrease in the number of corpora lutea was noted only at the 600 mg/kg/day dose level. No effects were observed in a supplemental study when TAK-375 was administered in a 2nd mating to males and no evidence of an effect on sperm count, motility or morphology was noted. A further study was undertaken to attempt to explain the difference in mortality observed in females between the Segment I fertility study and the 4-week reproductive toxicity study. The Sponsor believed this may be due to the different ages of the rats employed (11 weeks and 6 weeks, respectively) and the potential difference in exposure with these two ages were explored with doses identical to that used in the Segment I study. Results indicated that the older female rats used in the fertility study exhibited significantly higher plasma exposure to TAK-375 than their younger counterparts and this was considered to be the explanation for the mortality in the fertility study. Another supplemental study explored a different dose range in the Segment I study

(20, 60 and 200 mg/kg/day). Only irregular estrus cycles were exhibited in the 60 mg/kg/day group; no difference in the number of implants or live embryos was shown as in the initial study. The Sponsor does not provide an explanation for this difference between the studies. The ages of the animals, dosing duration of females, and other potential variables were similar. Toxicokinetics were not obtained in the 2nd study therefore the extent or adequacy of exposure cannot be determined. For this reason the initial study will be used for identification of NOAEL levels and risk assessment. The NOAEL for fertility and early embryonic development is considered to be 20 mg/kg/day as no effects were observed at this dose in either study. As toxicokinetic analysis was not completed for this study a body surface area conversion of the dose into mg/m² provides a margin of safety over the expected human exposure of 26-fold in a 65 kg individual.

Embryo-fetal development "Segment II" studies were conducted in rat and rabbit.

In the rat Segment II study, TAK-375 was administered at 10, 40, 150 or 600 mg/kg/day to pregnant 13-week old female rats from Day 6 – 17 of pregnancy and with resulting maternal and fetal effects compared to dams treated with vehicle and their fetuses, the latter assessed after cesarean section on Day 20 of gestation. Clinical signs were noted in dams treated with TAK-375 $\geq$ 150 mg/kg/day. Clinical observations at 150 mg/kg/day included salivation, transient reduction in body weight and on necropsy an increase in liver weight. At 600 mg/kg/day dams also had signs consisting of ataxia, decreased spontaneous movement, lying on one side, reduction in feces and salivation. Body weight was significantly reduced as was food consumption at this dose level. The 600 mg/kg/day group dams also exhibited increased liver weight and adrenal gland weight increase upon necropsy. Fetuses examined from treated groups demonstrated malformations including diaphragmatic hernia as well as increased number of lumbar vertebrae observed in fetuses from dams treated with $\geq$ 150 mg/kg/day when compared with fetuses from untreated dams. Variations in the 150 mg/kg/day group consisted of skeletal findings such as irregular lumbar rib shape and scapula. Fetuses of dams in the 600 mg/kg/day TAK-375 group demonstrated cysts on the external genitalia in addition to the increased incidence of diaphragmatic hernia and increased lumbar rib number noted in fetuses of the 150 mg/kg/day group. Variations in this group resembled those in the 150 mg/kg/day group with the additional observation of split sternbra. Therefore, the maternal, embryo-fetal, and teratogenic NOAEL are considered to be 40 mg/kg/day. A subsequent study to determine the maternal plasma exposure at the NOAEL dose of 40 mg for both TAK-375 and the active metabolite M-II determined the TAK-375 and M-II AUC₀₋₂₄ was 17,790 ng•h/mL and 10,772 ng•h/mL, respectively. Subsequent examination of the M-II metabolite in the rat involved intravenous administration of up to 30 mg/kg M-II to pregnant female rats over Days 6-17 of gestation. No maternal toxicity was noted including no effects on fertility parameters and no effects were observed on fetal body weight, sex ratio, % alive or external, visceral or skeletal abnormalities when compared to control litters.

The Segment II study in the Japanese White rabbit involved administration of TAK-375 at doses of 0, 12, 60 or 300 mg/kg/day by oral gavage to pregnant females over Days 6-18 of pregnancy. No adverse effects were noted with dose levels of $\leq$ 60 mg/kg/day. TAK-375 at 300 mg/kg/day produced clinical signs of toxicity and transient reduction in body weight gain without evidence of effects on the fetus including number alive at C-section, body weight, sex ratio, external, visceral or skeletal examination abnormalities. An apparent

increase in the observation of irregular lumbar rib was within historical control data ranges for this lab for which data was provided. The maternal NOAEL was identified at 60 mg/kg/day while the embryofetal teratogenicity NOAEL in the rabbit was > 300 mg/kg/day. A subsequent study to determine the plasma concentrations of TAK-375 and M-II in pregnant rabbits at the dose which were associated with the embryofetal NOAEL (300 mg/kg/day) demonstrated the AUC₀₋₂₄ for TAK-375 and M-II was 71,463 ng•h/mL and 23,847 ng•h/mL, respectively.

A Segment III study of pre- and postnatal development was conducted using Sprague-Dawley rats. TAK-375 was administered by oral gavage to pregnant dams (F₀ generation) from Day 6 of pregnancy through parturition to Day 6 of lactation at doses of 0 (vehicle), 30, 100 or 300 mg/kg/day. Dams, the F₁ offspring, and F₂ offspring were examined for effects of maternal/F₁ neonatal exposure to TAK-375 on clinical signs, body weight, food consumption, and abnormalities on necropsy. Reproductive potential was assessed for F₀ dams and F₁ offspring while F₁ offspring were tested for developmental, behavioral and learning deficits. In F₀ dams treated with TAK-375 or vehicle, no toxicities were observed with dosing at 30 mg/kg/day while at TAK-375 ≥100 mg/kg/day increased adrenal gland weight was found at necropsy and was also associated with occasional observations of reduced body weight and body weight gain during the lactation period. Effects noted on weight correlated with sporadic findings of reduced food consumption during the lactation period. At 300 mg/kg/day the body weight reduction was also noted during the pregnancy period as well as in the lactation period and was observed along with a reduction in food consumption. A minority of dams in the 300 mg/kg/day group demonstrated poor nest-building and impaired lactation causing the deaths of their entire litter within days of birth. No effects on reproductive performance were observed in F₀ dams with TAK-375 dosing up to 300 mg/kg/day, however. F₁ offspring in the 30 mg/kg/day group did not demonstrate abnormalities on any parameter. F₁ offspring in the 100 mg/kg/day litters had reduced body weight during weaning as well as after weaning compared with their control litter offspring. This reduction in body weight was more prominent in 300 mg/kg/day F₁ offspring which also had evidence of developmental delays including an increased latency to eruption of lower incisors, reduced rate of acquisition of the righting reflex, and altered emotional responses when compared to their control F₁ litter counterparts. Also observed in the F₁ offspring in the 300 mg/kg/day litters only were an increased incidence of diaphragmatic hernia. F₁ offspring selected for evaluation of reproductive potential by mating and generation of the F₂ generation did not find an effect of TAK-375 exposure on reproductive performance when compared with F₁ offspring in the vehicle-treated control group. No differences were seen in the F₂ generation between treatment groups. The NOAELs identified by the this reviewer were in accordance with that proposed by the Sponsor: The NOAEL(maternal toxicity) in the F₀ dams was identified at 30 mg/kg/day due to observations of reduced body weight and food consumption as well as increased adrenal gland weight with dosing of 100 mg/kg/day. The NOAEL (reproductive function) was identified at 100 mg/kg/day in the F₀ dams due to the alterations in nest-building and lactation observed in a minority of animals at 300 mg/kg/day. For the F₁ generation the NOAEL (developmental toxicity) was 30 mg/kg/day due to observations of body weight in the 100 mg/kg/day litters which extended to beyond the lactation period while at the highest dose level, 300 mg/kg/day, developmental delays and alterations in behavior could be observed as well as an increased incidence of diaphragmatic

hernia. Although not defined by the Sponsor, the F₂ NOAEL (developmental toxicity) was identified by this reviewer as 300 mg/kg/day to F₀ dams as no differences were seen between the F₂ litters of any treatment group when compared with F₂ litters of control group offspring.

Special toxicology:

Several toxicology studies were performed to ascertain the effects of TAK-375 administration on plasma concentrations of various hormones (melatonin and thyroid hormones in aged mice; in rats: thyroid hormones, thyroid stimulating hormone [TSH], corticosterone, adrenocorticotrophic hormone [ACTH], estradiol, prolactin, melatonin, dehydroepiandrosterone [DHEA], testosterone, follicle stimulating hormone, and lutenizing hormone [LH]) and selected target tissues (Harderian gland in mice, liver in rats) identified by 2-year carcinogenicity bioassays.

Aged mice (62-63 weeks of age) were administered TAK-375 by oral gavage at 3, 30, or 300 mg/kg/day for 4 weeks at the conclusion of which plasma hormone levels were assessed and Harderian gland melatonin concentration and histology examined. Mice administered ≥ 30 mg/kg/day exhibited significantly increased plasma melatonin levels 1 hr after TAK-375 administration (males: 4- and 44-fold increase at 30 and 300 mg/kg/day, respectively at 1 hr post-dose; females: 3- and 20-fold increase at 30 and 300 mg/kg/day, respectively) which was still observed at 12-14 hr after dosing (3-8-fold increase in males at 30 and 300 mg/kg/day, respectively; 2-fold in females at the 300 mg/kg/day dose level) Melatonin was also increased in the pooled Harderian glands of treated mice in males ≥ 30 mg/kg/day and at 300 mg/kg/day in females. No evidence was found for alterations in plasma thyroid hormone levels or histologic changes in the Harderian gland. No immunohistochemical evidence for an increase in proliferating cell nuclear antigen (PCNA) expression, indicative of cell proliferation, was observed in Harderian gland cells either at doses up to 300 mg/kg/day for 4 weeks.

Male and ovariectomized female rats were administered 0, 6, 20, 60 or 200 mg/kg/day TAK-375 for 1 week and plasma concentrations of LH, FSH, testosterone (males), prolactin (females), ACTH, corticosterone, T₄, T₃, and TSH were evaluated at the conclusion of the study. Treatment-related effects were not apparent on plasma LH, FSH, prolactin, TSH and T₃ levels at any dose. Plasma testosterone was decreased significantly in males at 200 mg/kg/day (-67% vs. controls) while in females at this dose level a reduction in circulating T₄ concentrations (-27%) was observed. Corticosterone was highly variable and showed a nonsignificant trend towards reduction with dose in males and a trend toward an increase in females. These trends were also reflected in the ACTH levels obtained from these groups.

Female rats were used for assessment of TAK-375 exposure on levels of circulating estradiol and prolactin. Rats were administered 0, 15, 60 or 250 mg/kg/day TAK-375 for 5 weeks with no resulting effect on plasma estradiol or prolactin levels though an increase in irregular estrus characterized by prolonged metestrus and decreased body weight was observed with dosing at ≥ 60 mg/kg/day. Decreased food consumption was additionally seen with the 250 mg/kg/day dose level. No histopathologic findings were noted at any dose level that appeared to be related to treatment.

Male rats were used for assessment of TAK-375 exposure on levels of circulating plasma melatonin and dehydroepiandrosterone (DHEA) and their livers evaluated microscopically after 4 weeks oral administration of 0, 15, 60, 250 or 1000 mg/kg/day TAK-

375. Consistent with findings in mice given TAK-375, plasma melatonin was significantly elevated (12- to 52-fold with increasing dose) at 1 hr post-dosing with TAK-375 levels $\geq$ 60 mg/kg/day. This elevation in plasma melatonin was maintained (4- to 5-fold increase) at 15 hr post-dosing in groups treated with $\geq$ 250 mg/kg/day TAK-375. DHEA and corticosterone were also both elevated at dose levels $\geq$ 250 mg/kg/day (2-3 – fold and 2 – fold, respectively) in these animals. Examination of the liver from rats treated with TAK-375 revealed a proliferation of smooth endoplasmic reticulum without evidence of peroxisome proliferation at the 1000 mg/kg/day dose level. PCNA immunohistochemistry did not reveal a significant difference in labeling of hepatocytes when TAK-375-treated groups were compared to their respective controls though this may be due to what the Sponsor suggests were unusually high control values caused by a minority of animals in the control group. Assessment of the treated groups appeared to show a dose-dependent increase in PCNA labeling suggestive of a proliferative response to increasing TAK-375 dose level but this could not be statistically demonstrated.

Finally, studies were done in the male rat to determine the effects of TAK-375 administration on plasma testosterone levels and circulating LH levels after 7 or 28 days of TAK-375 administration at 0, 250 or 1000 mg/kg/day. Although significant decreases in plasma testosterone were observed for up to 4 hr with dose levels $\geq$ 250 mg/kg/day and 5 hr with the 1000 mg/kg/day dose with daily administration for 7-days, no effect on plasma LH levels were noted over the 8 hr period for which these animals were followed. A subsequent study which examined the effects of 28 days of repeated dosing with the same dose levels of TAK-375 replicated the transient reduction in plasma testosterone ($\downarrow$ 2 – 7-fold vs. control) but additionally found a significant increase in plasma LH concentrations in the 1000 mg/kg/day group only at 4.5 hr and 24 hr ($\uparrow$ 1.6-fold at both timepoints) after the final dose given on Week 4. Interim analysis at 2 weeks did not reveal an effect on LH though the reduction in circulating testosterone was detected.

2.6.6.2 Single-dose toxicity

Study Title: Oral Single Dose Tox study of TAK375 in rats

Study No: M-11-16

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Conducting laboratory and location: Drug Safety Res Labs. Takeda Chemical Industries, Osaka, Japan.

Date of study initiation: Oct 23rd 1997

GLP compliance: Yes, FDA and Japan

QA- Report Yes (x) No ()

Dosing

species/strain: Sprague-Dawley rats

#/sex/group or time point: 5/sex/dose

age/weight: 6wks/169-190g m and 129-146g f.

satellite groups used for toxicokinetics or recovery: ND (not done)

dosage groups in administered units: 0, 200, 600, 2000mg/kg single dose.

route, form, volume. oral gavage to rats fasted for 5hrs: 10ml/kg; control rats received the vehicle: 0.5% methylcellulose solution.

Drug, lot# and % purity: M375-002; — pure.

Formulation/vehicle: drug in 5% methylcellulose (MC) as a suspension.

Observations and Times:

Clinical signs: pre-dose, 8x during the 1st 8hrs postdose & daily thereafter for the 14d observation period.

Body weights: predose and on days: 2,6,9, and 13 postdose.

Gross pathology: last day of the 14d observation period, all rats were killed and visceral organs examined macroscopically (the same was done for those that died).

Results:

Mortality and Clinical signs: no deaths in the 200 and 600mg/kg. Single f dosed 2g/kg died the 1st day after dosing. No clinical signs in rats dosed 200 or 600mg/kg except for a single f dosed 600mg/kg where it was slightly ataxic and showed a depressed touch-escape response; the rat was normal within 4hrs post dose. Signs in the 2g/kg gr included: decreased motor activity, depressed touch-escape response in all rats, prone position and ataxic gait in 4m and all f, decreased body temp. in 4f seen 0.5hr postdose. The following were seen in the single HDf that died in addition to the above signs: lying on side, respiratory depression 1-2hr postdose becoming moribund, lacrimation, salivation, twitches, later the next morning the rat was found dead.

Body weights: a decrease of 3.5-8% less than the control in mean wt noted only in HDm+f gr on d2 and in HDm on d6 (6% less than the control).

Necropsy: no findings in the animals that survived except for a liver hemia in 1f dosed 600mg/kg. In the f of HD that died, there were red scattered spots in lungs and stomach, distended stomach with white liquid assumed to be the drug material, and enlarged adrenals.

Summary and Conclusion:

The lethal dose in male rats is >2000mg/kg and that in females is between 600&2000mg/kg.

Study title: Intravenous Single Dose Toxicity Study of TAK-375 in Rats

Key study findings:

- Mortality observed at 60 mg/kg (3/5 males; 0/5 females). No mortality at lower doses.
- Common clinical signs in all dose groups including vehicle (DMSO) control consisted of a transient ↓locomotor activity, respiratory rate, touch-escape response; also pale skin, prone or lateral position and ataxic gait. Recovered in ≤ 20 mg/kg dosed animals within 5 minutes but lasted 30-60 minutes at 60 mg/kg.
- High dose 60 mg/kg treatment resulted in additional clinical signs of hypothermia observed at 30 minutes and 1 hr post-dose with full recovery by 2 hr observation point. Some evidence of mydriasis and skin incrustation at the high dose in a minority of animals.
- The NOAEL observed in this study appears to be < 6 mg/kg due to the clinical signs which are transient but nonetheless acutely adverse. Though the vehicle used in this study (DMSO, concentration not specified) may contribute to the observed effects as a follow-up study utilizing lower doses of TAK-375 as well as PEG400 30 w/v% as a vehicle did not note any clinical signs.

Study no.: M-11-78

Module: 4.2.3.1 Single-Dose Toxicity

Conducting laboratory and location: Drug Safety Research Laboratories
Pharmaceutical Development Division
Takeda Development Division
Yodogawa-ku, Osaka 532-8686, Japan

Date of study initiation: August 5, 1998

GLP compliance: Yes (FDA & Japanese requirements)

QA report: Yes (X) no ()

Drug, lot #, and % purity: TAK-375, Lot # M375-004, $\bar{c}$ $\bar{d}$ stability certified

Methods

Doses: Saline, Vehicle (Dimethylsulfoxide), TAK-375 at 6, 20 or 60 mg/kg

Species/strain: Rat — CD(SD)IGS

Number/sex/group or time point (main study): 5 rats/sex/group

Route, formulation, volume, and infusion rate: Intravenous via tail vein, Group 1: physiological saline; Group 2 DMSO, TAK-375 dissolved in DMSO as a 6%, 2% or 0.6% w/v solution administered as 1 mL/kg

Satellite groups used for toxicokinetics or recovery: None

Age: 6 weeks at start of dosing

Weight: ♂159-192g; ♀123-151g at start of dosing

Sampling times: Animals were observed for 14 days after dose administration

Unique study design or methodology (if any): 14-days after dosing animals were sacrificed by exsanguination from the abdominal aorta and vein under diethyl ether anesthesia. Visceral organs examined microscopically. No statistical analysis was conducted.

Results

Mortality: 3/5 males in 60 mg/kg group died within 1 minute after injection. No females died at this dose level or below.

Clinical signs: Examined for survival and clinical signs before dosing and 8 times during the first 6 hr after dosing and then once daily during the remainder of the 14-day observation period. Observation times were at 0, 5, 15 and 30 minutes after the i.v. administration of test article or control solutions followed by observations at 1, 2, 4, 6 hr post-dose and reports of observations on Day 1, Day 6 and Day 14 post-dose.

Saline produced no alterations in clinical signs while the DMSO vehicle produced effects in female rats consisting of transiently ↓locomotor activity, touch-escape response and respiration with prone position, pale skin and ataxic gait all observed in 2/5 female animals. No effects were observed in male rats. All findings were absent within 5 minutes post-dosing. TAK-375 effects were generally similar and increased with dose. TAK-375 at 6 mg/kg produced similar responses as DMSO (decreased activity, responsiveness, prone position, bradypnea, pale skin and ataxic gait) though these were also observed to occur in 2/5 male rats at this dose. Higher doses produced these responses in nearly all animals with the addition of mydriasis noted in 1/5 males at 20 mg/kg. All clinical signs were absent by the 15 minute observation. Dosing at the 60 mg/kg level produced similar responses to lower

doses but clinical signs were observed out to 1 hr post-dosing in surviving males and affected females with full recovery by 2 hr. Additionally, hypothermia was observed in surviving males and 3/5 females at 30 minutes and 1 hr post-dosing but was not seen at the 2 hr time-point. Crust formation at the injection site was seen in 1/5 females at the 6 and 14-day observation.

Table 1 Clinical signs - incidence of findings

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Test article	Dose (mg/kg)	No. of rats (M/F)	Findings	Minute				Hour			Day					
				0	5	15	30	1	2	4	6	14				
Saline Vehicle*	0	5/5	No abnormalities													
TAK-375	6	5/5	Hematuria	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
			Decrease in locomotor activity	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Depression of touch-escape response	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Prone position	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Bradypnea	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Pale skin	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Ataxic gait	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Hematuria	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TAK-375	20	5/5	Hematuria	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
			Decrease in locomotor activity	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Depression of touch-escape response	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Prone position	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Bradypnea	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Pale skin	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Ataxic gait	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Crust formation in the injection site	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2
TAK-375	60	5a/5	Hematuria	5.5	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
			Decrease in locomotor activity	5.5	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Depression of touch-escape response	5.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Prone or lateral position	5.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Bradypnea	5.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Pale skin	4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Ataxic gait	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Mydriasis	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TAK-375	60	5a/5	Hematuria	5.4	2.4	2.4	2.3	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
			Decrease in locomotor activity	5.4	2.4	2.4	2.3	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Depression of touch-escape response	5.4	2.4	2.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Prone or lateral position	5.4	2.4	2.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Bradypnea	5.4	2.4	2.2	2.2	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Pale skin	0.0	1.0	2.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Ataxic gait	0.0	0.0	0.0	2.3	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Hypothermia	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Crust formation in the injection site	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1			

* Dimethylsulfoxide, M. Male; F. Female; 0 minute: just after the injection; - not observed; + observed
 † 3 males died immediately after the injection.

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Body weights: Body weights taken prior to dosing and on Days 2, 6, 9 and 13 post-dosing.

No changes in body weight gain were observed in females in any dose group. No alteration was observed in males treated with the 6 mg/kg dose of TAK-375. The 20 mg/kg dose in males appeared to decrease body weight gain by 10% relative to vehicle control though the 60 mg/kg dose could not be adequately assessed for a dose-dependent relationship due to the deaths of 3/5 animals in this group.

- Food consumption: Not evaluated
- Ophthalmoscopy: Not evaluated.
- EKG: Not evaluated.
- Hematology: Not evaluated.
- Clinical chemistry: Not evaluated.
- Urinalysis: Not evaluated.

Gross pathology: Visceral organs assessed macroscopically at the end of the 14-day observation period. No alterations were noted.

- Organ weights: Not evaluated
- Histopathology: Not evaluated
- Toxicokinetics: Not evaluated

Study title: Intravenous Single-Dose Toxicity Study of TAK-375 in Rats (Supplemental Study)**Key study findings:**

- Purpose of this study was to determine a NOAEL level for TAK-375 in the acute i.v. administration setting in the rat which was not found in the previous study which had a low dose of 6 mg/kg.
- Single dose intravenous administration of TAK-375 at doses of 0.02 mg/kg (equivalent to the expected clinical dose), 0.2 mg/kg or 2 mg/kg did not produce abnormal clinical signs, or evidence of macroscopic abnormalities. Mild reduction in body weight gain was observed by the end of the study in males and females ($\downarrow 6\%$ ♂; $\downarrow 13\%$ ♀) which was reflected in a reduced body weight gain from the first observation on Day 2.
- NOAEL = 2 mg/kg i.v.
- Change in observations of clinical signs in the animals of the vehicle group between the two acute i.v. studies in rat due to the change in vehicle used (DMSO $\rightarrow$ PEG400) and may contribute to the lack of observed effects in this current study as well.

Study no.: M-11-347**Module 4.2.3.1 Single-Dose Toxicity****Conducting laboratory and location:**

Drug Safety Research Laboratories
 Pharmaceutical Research Division
 Takeda Chemical Industries, Ltd.
 Yodogawa-ku, Osaka JAPAN

Date of study initiation: 10/23/2001**GLP compliance:** Japan only**QA report:** Yes**Drug, lot #, and % purity:** TAK-375; Lot# M375-022; $\geq 98\%$ purity; Confirmed stability**Methods**

Doses: Control (Saline), Vehicle (30 vol% PEG400), TAK-375 dose levels of 0.02, 0.2 or 2 mg/kg

Group	Test article	Dosage level (mg/kg)	Dosage volume (mL/kg)	Concentration (w/v%)	No. of animals (Animal No.)	
					Male	Female
1	Control	0	2	0	5 (1M1-5)	5 (1F1-5)
2	Vehicle	0	2	0	5 (2M1-5)	5 (2F1-5)
3	TAK-375	0.02	2	0.001	5 (3M1-5)	5 (3F1-5)
4	TAK-375	0.2	2	0.01	5 (4M1-5)	5 (4F1-5)
5	TAK-375	2	2	0.1	5 (5M1-5)	5 (5F1-5)

Control: Saline, Vehicle: 30 vol% PEG400 solution

Species/strain: Rat — CD(SD)IGS

Number/sex/group or time point (main study): 5 rats/sex/group

Route, formulation, volume, and infusion rate: Intravenous (caudal vein), 2 mL/kg volume, doses formulated in 30 vol% PEG400. Infusions occurred over one minute.

Satellite groups used for toxicokinetics or recovery: N/A

Age: 6 weeks at study initiation

Weight: ♂171-205g; ♀137-170g

Sampling times:

Unique study design or methodology (if any): Statistical analysis not performed.

Results

Mortality: No deaths were observed at any dose level.

Clinical signs: Observations occurred prior to dosing and from infusion for the first 15 minutes post-administration, followed by observations at 30 minutes, 1, 2 and 4 hr after dosing followed by once a day in the morning during a 14-day post-dose observation period.

No abnormal clinical signs were observed in any treated group or control group animal.

Body weights: Weights were taken prior to dosing and on Days 2, 6, 9 and 13 post-dosing.

A mild decrease in body weight gain was observed in males and females administered the high dose (-6%, males; -13%, females). Females were more sensitive to this effect. End of study body weight gain reductions in HD males and females was due to a reduction in body weight gain at the first observation on Day 2 which was observed to be ↓8% in males and ↓57% in females vs. saline control body weight gain. Statistics were not performed in this study as previously indicated.

Food consumption: Not evaluated

Ophthalmoscopy: Not evaluated

EKG: Not evaluated

Hematology: Not evaluated

Clinical chemistry: Not evaluated

Urinalysis: Not evaluated

Gross pathology: Visceral organs assessed macroscopically at the end of the 14-day observation period.

No abnormal macroscopic findings were observed from any animal in any dose group.

Organ weights: Not evaluated

Histopathology: Not evaluated

Toxicokinetics: Not evaluated

Other: N/A

Summary & Conclusion:

Clinical signs were not observed with any dose level of TAK-375 in male and female rats. Reduction in body weight gain at the 2 mg/kg dose, especially in females, was moderate by the end of the study but was not observed in the prior intravenous study (Document # M-11-78) in females at any dose up to 60 mg/kg and in males at a dose of 6 mg/kg.

Study Title: Oral Gavage Escalating Dose Acute Tox study of TAK375 in monkeys

Study No: M-11-11

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Conducting laboratory and location: Drug Safety Res Labs, Takeda Chemical Industries, Osaka, Japan.

Date of study initiation: Oct 7th 1997

GLP compliance: Yes, FDA and Japan

QA- Report Yes (x) No ()

species/strain: cynomolgus monkeys

#/sex/group: 2/sex

age/weight: 3years and 9months: 3.35-3.70kg m and 2.96-3.49kg f.

satellite groups used for toxicokinetics or recovery: ND

dosage groups in administered units: 0, 2, 20, 200, 2000mg/kg escalating doses.

route, form, volume: oral gavage: 10ml/kg; control monkeys received the vehicle: 0.5% methylcellulose solution.

Drug, lot# and % purity: M375-002; [] pure.

Formulation/vehicle: drug in 5% methylcellulose (MC) as a suspension.

Observations and Times:

Clinical signs: predose and 3times/day predosing and at 1 and 6hr post dose on each day of dosing.

Body weights: predose, before dosing on days of administration, and once per wk after the last dose.

Food Intake: predose and during the observation period.

Body (rectal) Temp. and HR: from all monkeys predose and at 1&14d recovery period, and 3 times (before dose, 1&6hr postdose) on the days of dosing.

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Hematology & Clin Chemistry: on all monkeys, once predose, 1d after each dose and 14d after the last dose. Blood collected from the femoral vein.

Results:

Table below from the sponsor summarizes the results:

Animal	Cynomolgus monkeys, 3 years and 9 months of age					
Test article	0.5% MC	TAK-375				
Dosage level (mg/kg)	0→0→0→0	2→	20→	200→	2000	14 days after the last dose
Dosage volume (ml/kg)	10	10	10	10	30	
No. of animals (M:F)	2:2	2:2				
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0	0:0
Clinical signs						
Vomiting	-	-	-	slight-marked# (M:2,F:1)	marked# (M:2,F:2)	-
Salivation	-	-	-	-	slight-marked# (M:1,F:2)	-
Pale oral mucosa	-	-	-	-	slight (M:2,F:2)	-
Body weight	-	-	-	-	-	-
Food consumption	-	-	-	-	-	-
Body temperature	-	-	↓(M:2, F:2)	↓(M:2, F:1)	↓(M:2, F:1)	-
Heart rate	-	-	↓(F:1)	↓(M:1, F:1)	↓(M:1, F:2)	-
Hematology	-	-	-	-	-	-
Blood chemistry	-	-	-	-	↑ UN (M:1)	-
Conclusion	It is concluded that the effect level for acute toxicity is 200 mg/kg.					

→ No treatment-related effects, ↓: Decrease, #: Toxicologically significant changes

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Clinical signs occurred around 1 and/or 6hr postdose in the MD&HD. There was no drug effect on B.wt or food intake in any monkey. Mean B.temp decreased 1-1.7C in both males and females dosed 20mg/kg around 1-6hr postdose, in 2m and 1f dosed 200&2000mg/kg. In 1 male dosed 2000mg/kg, the temp normalized within 24hr but in 1m and 1f, the decrease remained for 8days postdose normalizing on d14 postdose. Heart rate was decreased by 30-50bpm in 1f dosed 20mg/kg, in 1m and 1f dosed 200mg/kg after 1-6hr postdose, and in 1m and 2f dosed 2g/kg at 1-6hr postdose.

Summary and Conclusion:

Oral gavage administration of TAK375 to monkeys did not cause death upto 2g/kg. Clinical signs at the 2g/kg dose were similar to but more frequent and more severe than those seen in the 200mg/kg gr. Clinical signs included vomiting, salivation, and pale oral mucosa; vomiting occurred several times after dosing and was considered to be of toxicological significance. Body temp was reduced 1-1.7C between 1-6hr postdose for all 3 dose grs. In the HD, in some animals temp remained below normal untill day 14 of recovery period when it was normalized. HR was also reduced but recovered within 24hr of dosing. These 2 effects are common pharmacological effects of melatonin. Therefore, TAK375 was well tolerated after a single oral dose to the monkey upto 2g/kg.

2.6.6.3 Repeat-dose toxicity

Study title: Preliminary Two-Week Oral Gavage Toxicity Study of TAK-375 in Rats

Key study findings:

- TAK-375 administered by oral gavage to rats for 2 weeks with dosing up to 2000 mg/kg/day produced: excessive mortality, clinical signs including ataxia and chromaturia, reductions in bodyweight gain, clinical chemistry alterations

indicating liver and kidney effects. Gross and histopathologic assessment of organs revealed alterations in liver, adrenal gland, thyroid gland and thymus weight and structure.

- A NOAEL in this study is identified at 200 mg/kg/day while the NOEL is considered to be the low dose of 30 mg/kg/day. Evidence of minimal centrilobular hepatocellular hypertrophy indicative of liver enzyme induction and increased liver weight is seen at the NOAEL.

Study no.: M-11-00610

Module: 4.2.3.2 Repeat Dose Toxicity

Conducting laboratory and location: Drug Safety Research Laboratories
Takeda Chemical Industries, Ltd.
Osaka, Japan

Date of study initiation: October 22, 1997

GLP compliance: No

QA report: No

Drug, lot #, and % purity: TAK-375, Lot #M375-002, [] purity

Methods

Doses: Vehicle (0.5% methylcellulose solution), TAK-375: 30, 200, 600 or 2000 mg/kg/day

Species/strain: Rat CD(SD)IGS

Number/sex/group or time point (main study): 4 rats/sex/group

Route, formulation, volume, and infusion rate: Oral gavage, TAK-375 dissolved in 0.5% methylcellulose, 10 ml/kg solution administered

Satellite groups used for toxicokinetics or recovery: TAK-375 TK groups (30, 200, 600 and 2000 mg/kg/day); 4 rats/sex/group.

Age: 6 weeks at initiation of study

Weight: ♂164-189g; ♀130-151g

Sampling times: Plasma concentrations of TAK-375 were assessed in satellite groups at 0.5, 1, 2, 4, 8 and 24 hr after the 1st administration of the treatment article. No assessment was made at the end of the 2 week dosing period.

Unique study design or methodology (if any): It is unclear whether rats were housed with reversed lights on/lights off timing.

Results

The sponsor provided the following tabulated summary of the study:

Animal	D(XSD)IGS rats aged 6 weeks at the start of treatment				
Test article	Control	TAK-375			
Dose (mg/kg/day)	0	30	200	600	2000
No. of animals (M:F)	4:4	4:4	4:4	4:4	4:4
Mortality (M:F)	0:0	0:0	0:0	0:0	1:3 a)
Clinical signs	—	—	—	Ataxic gait # (M:2, Day 0), Chromaturia	Animals that survived (M:3, F:1): Ataxic gait # (Day 0), Chromaturia Animals that died or were sacrificed in extremis (M:1, F:3): Ataxic gait #, Lying on side #, ↓ Abdominal tone #, Bradypnea #, ↓ Body temperature #, Salivation #, ↓ Feces
Body weight b)	—	—	—	↓ # (F)	↓ #
Food consumption b)	—	—	—	toward ↓ (F)	↓ #
Ophthalmoscopy b)	—	—	—	—	—
Urinalysis b)	—	—	—	—	—
Hematology b)	—	—	—	—	↓ MCH • MCV # (M), ↓ WBC • Ret # (F)
Blood chemistry b)	—	—	—	↑ TP • Ca # (M), ↑ Tcho • TG # (F)	↑ Tcho • TG • UN • Cre • TB • ALT • Ca #, ↓ A/G • Na #, ↑ TP # (M), ↓ Glu • CK # (M), ↑ AST # (F)

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—: No treatment-related abnormalities, †: Increase, ‡: Decrease, #: Toxicologically significant changes
 Day 0 is the first dosing day.
 a): Two females were sacrificed in extremis about 24 hours after the 1st dose. One male died and 1 female was sacrificed in extremis about 4 hours after the 2nd doses.
 b): In the animals that died or were sacrificed in extremis, these examinations could not be carried out or the data were excluded from the tabulated summary.

Mortality: Two females in the 2000 mg/kg/day group were sacrificed after about 24 hr of the 1st dose while one female was sacrificed at about 4 hr after the dose on Day 2. One male in the 2000 mg/kg/day dose was sacrificed in extremis also about 4 hr after the high dose on Day 2. Common observations were ataxic gait, lateral recumbency, bradypnea and hypothermia which began within 1 hr of dose administration. All remaining animals in the 2000 mg/kg/day group survived to the conclusion of the study and no animals in any other dose group demonstrated mortality.

Clinical signs: Observation times were not described in methodology but table suggests this was done daily.

Clinical signs were apparent in males and females receiving a dose of 600 mg/kg/day beginning on Day 0 of study. Males (2/4) demonstrated an ataxic gait while females did not have this response at this dose level. Chromaturia classified as brown or red was observed in all males and females in this dose group (8/8), and this effect was observed intermittently in both males and females with females apparently more sensitive to this effect. 2/4 males were only noted to have this response on Day 3 of the 2-week study. Chromaturia was characterized as being of marked severity in both genders. At 2000 mg/kg/day these effects were observed as well as additional signs in one male of indicative of a prone position (lying on one side, decreased locomotion), hypothermia, bradypnea, decreased feces, salivation and decreased abdominal tone. These effects in this male animal were observed on Day 0 or 1

only and this animal was sacrificed. Females demonstrated similar clinical signs in greater numbers over the same time period and required the termination of ¾ females in this dose group. No clinical signs were observed in the low dose 200 mg/kg/day group.

Body weights: Body weights were taken pre-dose on Day 0, as well as on Days 3, 7, 10 and 14 of the study.

A dose- and gender-dependent reduction in bodyweight gain was observed as depicted in the following table:

Effect of TAK-375 on Body Weight Gain vs. Control Animals					
	Control	TAK-375 dose (mg/kg/day)			
		30	200	600	2000
♂	-	+5%	-4%	-5%	-47% [@]
♀	-	-15%	-19%	-33%	-67% [@]

[@], data obtained from 3 surviving males or 1 surviving female at this dose level

Male rats did not demonstrate reductions in bodyweight gain until reaching the highest dose tested while female rats proved more sensitive with the lowest dose of 30 mg/kg/day TAK-375 producing a ↓15% bodyweight gain compared with control animals and increasing in severity with dose escalation.

Food consumption: Food consumption was analyzed as grams/animal/week, therefore only two observations were made during the course of the study.

TAK-375 administration did not affect food consumption in male rats until reaching the 2000 mg/kg/day dose level at which consumption ↓31%, mirroring the bodyweight gain reduction observed in this group. Food consumption improved slightly during week 2 for this group but was still ↓20% relative to control rats. Female rats did not demonstrate any alteration in food consumption at doses ≤ 200 mg/kg/day. At 600 mg/kg/day females exhibited an approximately 12% decrease in food consumption relative to control rats for both weeks. The sole surviving female rat administered 2000 mg/kg/day showed a decrease of 26% and 37% versus control animals over weeks 1 and 2, respectively.

Ophthalmoscopy: Evaluation performed at the conclusion of the study. No treatment-related effects were observed and although occasional findings were noted, these were not dose-dependent or found in more than one animal.

EKG: Not evaluated

Hematology: Blood was taken at the conclusion of the study for analysis. Mean cell hemoglobin and mean cell volume was decreased in males in the 2000 mg/kg/day group while in the surviving female at this dose level there was evidence of decreased leukocytes and reticulocytes.

Clinical chemistry: Blood was taken at the conclusion of the study for analysis. Administration of TAK-375 appeared to alter a number of clinical chemistry parameters

including decreases in albumin/globulin ratio and plasma glucose. Increases were found in total cholesterol, a finding which was dose-dependent, triglycerides, BUN, creatinine, Total bilirubin and ALT. These findings were most pronounced or were exhibited exclusively at the 2000 mg/kg/day dose level in male rats. The sole surviving female at the 2000 mg/kg/day dose appeared to exhibit similar findings. Rats treated with ≤ 200 mg/kg/day TAK-375 did not appear to demonstrate these alterations.

Urinalysis: Urine was obtained on Day 8 and the analysis did not detect differences between treated groups and control animals.

Gross pathology:

Organ weights: Organs weighed included the following: brain, heart, lungs, liver, kidneys, spleen, pituitary gland, adrenal glands, and thymus. Males additionally had the testes, ventral prostate and seminal vesicles weighed while female rats had the ovaries weighed.

Body weight was decreased in males and females at the high dose although this was only significant in males due to only 1/4 females surviving to termination at the 2000 mg/kg/day dose level. Notable increases in liver weight were observed in both males and females and appeared to be dose-dependent and began to appear a 200 mg/kg/day (relative to body weight); 600 mg/kg/day when absolute values were used. All other changes appeared to occur solely at the highest dose of 2000 mg/kg/day and included increased adrenal gland weight, decreased heart weight (females only), and decreased ventral prostate and seminal vesicle weight (males only). Changes in brain weight were inconsistent between species and when expressed as both absolute and relative changes. See table below.

Significant Organ Weight Changes					
		Dose Level (mg/kg/day)			
		Males		Females	
Organ	Change	Absolute	Relative	Absolute	Relative
Adrenal glands	↑	2000	2000	2000	2000
Liver	↑	600 2000	200 600 2000	600 2000	200 600 2000
Brain	↑↓	-	2000 (↑)	2000 (↓)	-
Heart	↓	-	-	2000	2000
Ventral prostate	↓	2000	2000		
Seminal Vesicle	↓	2000	2000		

Histopathology: Adequate Battery: yes (), no (X); only selected tissues were examined: Liver, kidney, spleen, thymus, adrenals, thyroid, brain (ncluding pars tuberalis and basilar artery), pituitary gland, testes, prostate, epididymides, seminal vesicle, ovaries, pineal gland, eye, Harderian glands, jejunum, colon, mammary glands, and lung.

Peer review: yes (), no (X)

Microscopic analysis of selected tissues revealed the following:

Microscopic Findings from 2-Week Oral Toxicity Study in the Rat										
ORGAN SYSTEM	Males Dose Administered (mg/kg/day)					Females Dose Administered (mg/kg/day)				
	C	30	200	600	2000	C	30	200	600	2000
# Examined	4	4	4	4	4 [†]	4	4	4	4	4 [†]
Liver										
Centrilobular hypertrophy	0	0	4	4	4	0	0	4	4	2
Focal necrosis	0	0	0	0	2	0	0	0	0	0
Intracytoplasmic eosinophil	0	0	0	0	3	0	0	0	0	1
Adrenal gland										
Vacuolization, cortex	0	0	0	1	3	0	0	0	0	1
Thyroid gland										
Follicular cell hypertrophy	0	0	0	3	2	0	0	0	0	0
Spleen										
Hematopoiesis	0	0	0	0	0	0	0	0	0	3
Thymus										
Necrosis, lymphocyte	0	0	0	0	1	0	0	0	0	4
Epididymidis										
Cellular debris, lumen	0	0	0	0	1					n/a
<i>All other tissues were judged normal</i>										

C, control group; [†], 1 male found dead; [‡], 3 females sacrificed moribund

The liver appeared to be a significant target organ for TAK-375 with centrilobular hypertrophy occurring in both males and females beginning at 200 mg/kg/day with increasing severity with increasing dose. Focal necrosis of hepatocytes was noted in males at 2000 mg/kg/day only and was not found in females. Dose-related vacuolization of the adrenal gland was found in both males and females with males appearing more sensitive. Follicular cell hypertrophy was apparent in males treated with doses $\geq$ 600 mg/kg/day and in the surviving female given 2000 mg/kg/day. Evidence of extramedullary hematopoiesis was found in female rats, including those sacrificed moribund, at 2000 mg/kg/day but was absent in males. Thymic lymphocyte necrosis was observed only at the highest dose of 2000 mg/kg/day in males and females with females being more sensitive.

Toxicokinetics:

Toxicokinetic assessment of blood samples revealed an approximately 2-fold greater exposure in females relative to males in both peak concentrations and AUC₀₋₂₄. Exposure increased in a dose-dependent manner but not quite dose-proportionally (variable). T_{max} was < 1 hr at doses $\leq$ 600 mg/kg/day but increased to 3-6 hr with the 2000 mg/kg/day dose level. See sponsor's table below:

Table 1 Toxicokinetic parameters for TAK-375 in rats

Dose (mg/kg)	Male			Female		
	Tmax (h)	Cmax (µg/mL)	AUC _{0-24h} (µg·h/mL)	Tmax (h)	Cmax (µg/mL)	AUC _{0-24h} (µg·h/mL)
30	0.50 (0.00)	1.85 (0.43)	2.4 (0.8)	0.67 (0.29)	4.06 (1.42)	4.9 (1.1)
200	0.50 (0.00)	10.81 (3.23)	57.0 (8.2)	0.50 (0.00)	20.79 (4.49)	120.8 (51.6)
600	0.50 (0.00)	17.71 (2.38)	114.8 (20.4)	0.67 (0.29)	34.27 (7.95)	203.5 (58.3)
2000	3.00 (4.33)	40.13 (28.53)	643.2 (722.3)	8.00 *	91.73 *	1615.6 *

Mean (S.D.). *: N=2.

Summary & Conclusions

Oral administration of TAK-375 to rats for 2 weeks with dosing up to 2000 mg/kg/day produced excessive mortality, clinical signs involving ataxic gait and chromaturia with dosing $\geq$ 600 mg/kg/day and reductions in bodyweight gain in males at the highest dose and females in all dose groups, partly explained by reductions in food consumption. Mild hematologic effects were observed at 2000 mg/kg/day but clinical chemistry parameters were significantly altered A/G ratio, and plasma glucose. Dose dependent increase in cholesterol was observed. Increases were also found in triglycerides, BUN, creatinine, total bilirubin and ALT at TAK-375 dose increased or at the 2000 mg/kg/day level only. The liver appeared to be the most sensitive organ for toxicity with increased liver weight observed with dosing $\geq$ 200 mg/kg/day which correlated with the microscopic findings of centrilobular hypertrophy. Examination by electron microscopy revealed an increase in smooth endoplasmic reticulum which supports an explanation of a drug-related enzyme induction though inclusion bodies were considered to be a sign of toxicity. Focal necrosis of liver hepatocytes, described as minimal in severity, was noted in two male rats at the 2000 mg/kg/day TAK-375 level. Increased adrenal gland weight was noted in both males and females and correlates with adrenal cortex vacuolation found on histologic analysis. Decreased thymus weights observed with 2000 mg/kg/day dosing in both males and females was on microscopic assessment found to represent lymphocytic necrosis and is a probable response to stress encountered at this dose.

Thus, target organs of TAK-375 in a 2-week rat study appear to be the CNS, liver, adrenal gland, thyroid gland and thymus with liver demonstrating greatest sensitivity. The greater toxic responses observed in females appears to be explainable by the nearly 2-fold increase in exposure to TAK-375 found in the toxicokinetic analysis of plasma in satellite groups. A NOAEL would appear to exist at 200 mg/kg/day with a NOEL of 30 mg/kg/day.

Study title: Preliminary Two-Week Oral Gavage Toxicity Study of TAK-375 in Rats (Supplemental Study)

Key study findings:

- No biologically significant differences in the toxicity or toxicokinetic profile were observed between this study in which TAK-375 was administered in the evening (approximately 5 p.m.) and the previous study (M-11-00610) in which it was administered in the morning (approximately 9 a.m.).
- It is unclear whether rats were housed with reversed lights on/lights off timing.

Study no.: M-11-00611

Module: 4.2.3.2 Repeat Dose Studies

Conducting laboratory and location: Drug Safety Research Laboratories
Takeda Chemical Industries, Ltd.
Osaka, Japan

Date of study initiation: December 25, 1997

GLP compliance: No

QA report: No

Drug, lot #, and % purity: TAK-375; Lot # M375-002, [] pure

Methods

Doses: Vehicle (0.5% methyl cellulose), TAK-375: 30, 200 or 600 mg/kg/day

Species/strain: Rat/CD(SD)IGS

Number/sex/group or time point (main study): 4 rats/sex/group

Route, formulation, volume, and infusion rate: Oral gavage, TAK-375 formulated in 0.5% methylcellulose and administered as 10 ml/kg/day

Satellite groups used for toxicokinetics or recovery: 3 groups, 4 rats/sex/group

Age: 6 weeks at study initiation

Weight: ♂: 168-189g; ♀: 128-155g

Sampling times:

Unique study design or methodology (if any): This study utilized TAK-375 administration during the evening (approximately 5:00 p.m.) for 2 weeks whereas the previous study, identical in other respects, utilized dosing in the morning (time not specified).

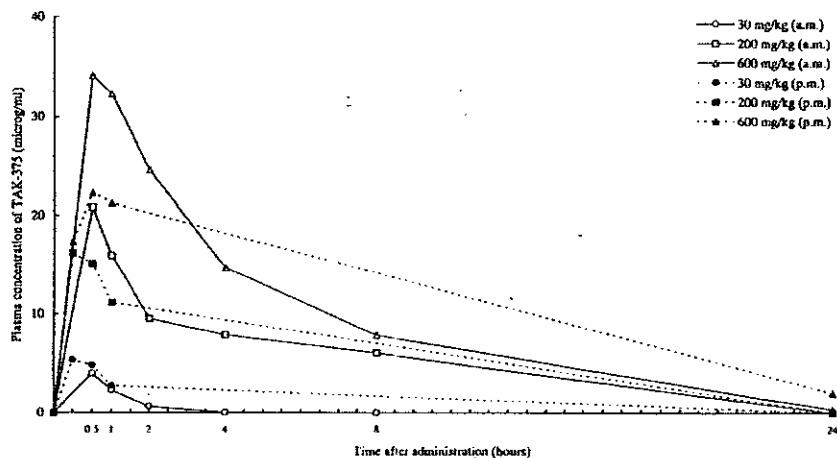
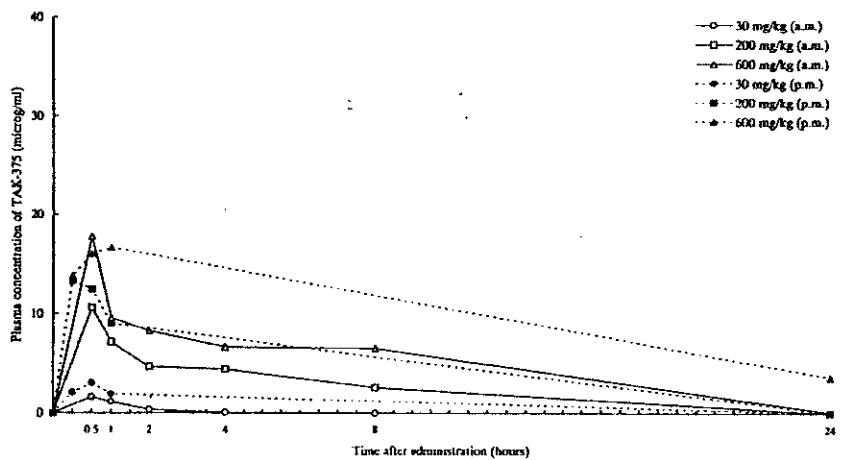
Results

The sponsor provided the following table comparing M-11-00610 (morning dosing) versus this study M-11-00611 (evening dosing):

Test article	TAK-375					
	30		200		600	
Dosage level (mg/kg/day)	9:00 a.m.	5:00 p.m.	9:00 a.m.	5:00 p.m.	9:00 a.m.	5:00 p.m.
Clinical signs	—	—	—	↑ Locomotor activity # (F:1), Licking # (F:1), Salivation (M:2, F:3)	Ataxic gait # (M:2), Chromaturia (M:4, F:4)	Ataxic gait # (M:1, F:1), Chromaturia (F:2), Licking # (F:1), Salivation (M:4, F:3)
Body weight	—	—	—	—	↓ # (F)	toward ↓ (M)
Food consumption	—	—	—	—	toward ↓ (F)	toward ↓
Blood chemistry	—	—	—	↑ TP • Alb (M), toward ↑ Tcho • TG (F)	↑ TP • Ca # (M), ↑ Tcho • TG # (F)	↑ TP • Alb (M), ↑ Tcho • TG # (F)
Organ weight	—	—	↑ Liver	↑ Liver	↑ Liver	↑ Liver, ↑ Adrenals # (F)
Histopathology	—	—	Liver: Hypertrophy of hepatocytes, Thyroids: Hypertrophy of follicular cells # (F:1)	Liver: Hypertrophy of hepatocytes	Liver: Hypertrophy of hepatocytes, Thyroids: Hypertrophy of follicular cells # (M:3, F:1), Adrenals: Vascuolization in cortical cells # (M:1)	Liver: Hypertrophy of hepatocytes, Adrenals: Vacuolization in cortical cells # (M:1, F:1)

—: No treatment-related abnormalities, ↑: Increase, ↓: Decrease, #: Toxicologically significant changes

In addition, the sponsor provided the following graph depicting the toxicokinetic profile of rats administered TAK-375 in the morning versus in the evening (male rats, top; female rats, bottom):



Study Title: 4 week Oral Gavage Tox study of TAK375 in rats

Study No: M-11-27

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Conducting laboratory and location: Drug Safety Res Labs, Takeda Chemical Industries, Osaka, Japan.

Date of study initiation: Mar 11th 1998

GLP compliance: Yes, FDA and Japan

QA- Report Yes (x) No ()

species/strain: Sprague-Dawley rats

#/sex/group: 10/sex/dose with additional 4/sex/dose for TK

age/weight:

dosage groups in administered units: 0, 10, 40, 150, 600mg/kg/d administered in the *morning*.

route, form, volume: oral gavage; 10ml/kg; control rats received the vehicle: 0.5% methylcellulose solution.

Drug, lot# and % purity: M375-002, [] pure.

Formulation/vehicle: drug in 5% methylcellulose (MC) as a suspension.

Observations and Times:

Clinical signs: once in the am predose and twice/day (predose and between 0-2hr postdose); detailed exam of clinical signs was done 2x per week during dosing, 3times each day (predose,

0-2hr postdose, and 3-5hr postdose).

Body weights: predose, before dosing on 1st day of dosing, d3, d7, and 2x per wk thereafter.

Food Intake: once a week on the day of feeding, the wt of the diet given to each animal and that of the diet remaining were determined and food intake for the week was calculated.

Water Intake and Urine output: both parameters taken from 5 rats/sex/dose on d22 of dosing. Urine output was collected over 24hr.

Ophthalmoscopy: predose and on d27 of dosing on 5/sex/dose; both direct and indirect measurements were done.

Urinalysis: d22 of dosing in 5/sex/dose.

Hematology and Clin Chemistry: from all animals (fasted for 20hrs*) after the last dose.

* 20hr seems a long time without food in the rat, prolonged period of no food can by itself alter clinical parameters. Fasting is usually done overnight.

Liver Enzyme Activity: liver samples were collected from 5/sex/dose.

Activity of liver UDP-glucuronyltransferase and sulfotransferases for triiodothyronine, T3, or 4-nitrophenol: liver samples from control and 600mg/kg gr were collected at end of study, microsomes and cytosol samples were prepared according to standard procedures.

Gross morphology and Organ wts: on the day after the last dose, all animals from all drug grs were examined.

Histopath: from all animals but those processed and stained with H&E were from the control and HD rats as well as the following tissues from the other 3 dose grs: liver, adrenals, thyroid, ovaries, and vagina.

TK: plasma levels of unchanged drug were determined in blood collected after the 1st and 29th dose in a satellite gr from 3/sex/dose. Samples were collected predose (for the 29th dose only), 15, 20min, 1, 3, 6, and 24hr postdose.

Results:

Tables below from the sponsor present summary of findings:

Four-Week Oral Gavage Toxicity Study of TAK-375 in Rats (Study No. 2699/SU)

Animal	CD(SD)IGS, 6 weeks of age				
Test article	Control*	TAK-375			
Dosage level (mg/kg/day)	0	10	40	150	600
Dosage volume (mL/kg)	10	10	10	10	10
No. of animals ¹⁾ (M:F)	10:10	10:10	10:10	10:10	10:10
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0
Clinical signs	—	—	—	Salivation	Salivation, Chromaturia
Body weight	—	—	—	—	↓ Gain (M; Dry 28)
Food consumption	—	—	—	—	↓ (F; Week 1, 2)
Urine output, Water intake ²⁾	—	—	—	—	—
Ophthalmoscopy ²⁾	—	—	—	—	—
Urinalysis ²⁾	—	—	—	—	—
Hematology	—	—	—	—	↓ Retic (M), ↑ Retic (F)
Blood chemistry	—	—	↑ TCCho (F)	↑ TCCho (F), ↑ TBil (F)	↑ TPp-Cat, ↑ Alb (M), ↓ ALP-K (M), ↑ TCCho+TG+ALT (F), ↓ ALG-TBil-AST (F)
Blood T3, T4 and TSH levels	—	—	—	↑ T3 (F)	↑ T4 (M), ↑ T3 (F), ↓ TSH (M), ↓ TSH (F)
Activity of hepatic drug-metabolizing enzymes ²⁾	—	—	—	↑ Aniline hydroxylase (M)	↑ Amino pyrimidine-demethylase, Aniline hydroxylase
Activity of hepatic UGT and ST for T3, T4 or 4-nitrophenol	—	NE	NE	NE	↑ UGT for T4 - UGT for 4-nitrophenol

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Animal	CD(SD)IGS, 6 weeks of age				
Test article	Control*	TAK-375			
Dosage level (mg/kg/day)	0	10	40	150	600
Gross pathology	—	—	—	Liver: Dark discoloration (F:2) Adrenals: Enlarged (F:2)	Liver: Enlarged (M:9,F:4) Dark discoloration (F:10) Adrenals: Enlarged (M:3,F:7)
Organ weights	—	—	↑ Thyroid (M)	↑ Thyroid (M), ↓ Adrenals (F)	↑ Liver, ↑ Thyroid (M), ↓ Adrenals (F)
Histopathology	—	—	Adrenals: Vacuolation of cortical cells (M:2)	Liver: Hypertrophy of hepatocytes (M:3,F:2) Adrenals: Vacuolation of cortical cells (M:4) Ovary: Vacuolation of interstitial cells (F:2)	Liver: Hypertrophy of hepatocytes (M:10,F:10) Adrenals: Vacuolation of cortical cells (M:9,F:8) Ovary: Vacuolation of interstitial cells (F:3)
Concentrations of unchanged TAK-375 in plasma ³⁾		(M:F)	(M:F)	(M:F)	(M:F)
Tmax (h)	1a: 0.3-0.3 29a: 0.4-0.3	0.3-0.3 0.3-0.5	0.3-0.3 0.3-0.5	0.3-0.4 0.3-2.3	0.4-0.7 4.1-0.6
Cmax (µg/mL)	1a: 0.35-0.49 29a: 2.66-1.27	2.66-1.27 2.35-7.05	2.99-4.99 4.2-9.5	11.48-14.23 4.19-6.08	19.69-30.86 6.11-37.71
AUC0-24h (µg·h/mL)	1a: 0.3-0.3 29a: 1.4-1.0	0.3-0.3 4.0-10.2	4.2-9.5 4.0-10.2	32.0-93.9 23.2-33.4	142.4-263.8 72.0-204.5
Conclusions	Non-toxic dose: 10 mg/kg/day				

* 0.5 w/v% methyl cellulose solution, — No treatment-related effects, † Increase, ‡ Decrease, # Toxicologically significant changes, NE: Not examined, M: Male, F: Female, UGT: UDP-glucuronosyltransferase, ST: Sulfotransferase
 1) Additional 4 animals/sex/dose were used as satellite groups for toxicokinetics.
 2) Conducted in 5 animals in each group.
 3) Mean values of 3 males and 3 females

There were no deaths in any gr. Clinical signs were limited to the 2 high doses and included salivation and brown chromaturia (only in 600mg/kg gr.). The salivation was attributed to the bitter taste of the drug and not to a direct drug effect. The sponsor also suggested that the *chromaturia was caused by the metabolites in urine and not the parent because there were no histopath findings in kidneys or urinary bladder and no changes in urinalysis parameters.* These are assumptions and a direct drug effect can not be excluded. Mean wt was reduced only at end of study d28 in 600mg/kg males. Although food intake was unaffected in the HDm, it was decreased in HDf during the 1st 2wks of dosing. Also a statistically significant decrease in food was seen in females dosed 40mg/kg during the 2nd wk of dosing. No drug related findings were seen in water intake, urine output, ophthalmoscopy, hematology, or urinalysis. Total cholesterol (TC) was increased in females dosed ≥ 40 mg/kg. In HDm+f both total protein and Ca levels were elevated and in f only, TG levels were increased. Activity of hepatic aniline hydroxylase was increased in 150mg/kg male gr and in both sexes of 600mg/kg gr., activity of aminopyrine N-demethylase was also increased in 600mg/kg rats. Blood levels of T3 were reduced in females dosed 150 & 600mg/kg, T4 levels increased in males dosed 600mg/kg, changes in TSH were inconsistent. Thyroid mean wt was increased in males dosed ≥ 40 mg/kg, mean wt of the adrenals of females dosed 150&600mg/kg, and mean liver wt was increased in HD rats as well as in 150mg/kg gr. No histopathology was seen for the thyroid. Centrilobular hypertrophy was seen in 5/sex of 150mg/kg gr and in all rats dosed 600mg/kg. Vacuolation of the adrenals (fine ones seen in the zona fasciculata) were seen in 2 males dosed 40mg/kg, 4males in 150mg/kg, and 9m/8f in the 600mg/kg gr. Vacuolation of ovarian interstitial cells was seen in 2f dosed 150mg/kg and 5f dosed 600mg/kg. Tmax following the 1st dose for all drug grs and for the 29th dose for 10&40mg/kg grs ranged between 0.3-0.7hr; Tmax tended to increase (prolonged) in the 150 and 600mg/kg grs on d29 of dosing. Mean concentration and exposure increased with increase in dose but the increase was more than proportional between 10-150mg/kg and below proportional between 150 and 600mg/kg grs for both parameters. Female values were higher than the male values. Mean max concentration in the 150&600mg/kg grs were lower for both sexes on d29 than those measured on d1. However, max concentrations for the 10&40mg/kg grs on d29 were either comparable or higher than those on d1.

4-week oral gavage tox study of TAK-375 in rats with 13 wk recovery period/
M-11-110/study# 2784/SU

Conducting lab: Takeda, Japan
GLP: Yes/Japan & FDA
QA report: Yes
Initiation/Termination dates: Aug 1998/Dec 1998
Drug lot#/purity: M375-006 []
Strain/# per sex per dose: SD rats: 30/sex/dose; additional 10/sex/dose each for the 4wk and 13wk recovery periods.
Doses & vehicle: 600mg/kg d; the control group received the vehicle: 0.5%w/v methylcellulose. Administration volume: 10ml/kg.
Route/Duration: daily oral gavage for 4wks
B.wt/age at initiation: 6wks/179-204g males and 131-157g females

Organ wts: absolute and relative wts of the following organs were determined at necropsy and both recovery periods: brain, thymus, heart, lungs, liver, spleen, kidneys, testes/ovaries, pituitary, adrenals, and ventral prostate.

Gross necropsy: standard exam of external appearance and organs/tissues of rats at end of dosing and both recovery periods.

Histopath: the following organs/tissues from all animals at end of dosing and wk4 recovery were isolated and fixed in 10% buffered formalin: brain, heart, lungs/bronchi, liver, kidneys, spleen, testes/ovaries, ventral prostate, thymus, pituitary, adrenals, and thyroids. The following organs/tissues from all rats were stained with H&E and examined by LM:

End of dosing: liver, thyroid, adrenals, and ovaries.

End of 4wk recovery: liver, thyroids, adrenals, ovaries, and spleen.

End of 13wk recovery: adrenals.

Results:

Mortality: no deaths.

Clinical signs: during dosing: salivation (26/30m and 22/30f) and chromaturia (30/30m and 27/30f); incidence increased with repeated dosing. Only chromaturia remained during the 1st wk of recovery (17/30m and 16/30f) and was absent from recovery wk2 onward.

B.wt: slight but significant decrease in wt gain was seen in dosed males; no effect at either recovery period. These decreases were seen during dosing days 3, 10, 21, 24, and 28 and ranged between 2-4.4% less than the control values.

Food intake: slight but significant decrease in intake in males during 1st wk of dosing (5% less than control), and in females throughout dosing period (5-8% less than control). Slight but significant increase (7.7% more than control), in intake in females during 1st wk of recovery; intake was comparable to control thereafter.

Hematology: the following reached statistical significance relative to the control values:

	D29	R30	
RBC	↓ 1% m/3.4%f*	↓ 4.3% m	
HcT	↓ 2.3% m	-	
MCHC	↑ 1% m	/↓ 1.1% f	-
% retic	↑ 22.7% m	-	
plateletes	↑ 14% f	-	

* not statistically significant. D29 is end of dosing period; R30, end of 1st recovery period. The hematology findings were not considered adverse effects since they were small and/or within historical range.

Clinical Chemistry: the following parameters reached statistical significance and were affected in both sexes:

Dosing period:

Total proteins: ↑ 7% over control for both sexes
 Total cholesterol: ↑ 1.6 fold in m and 2.2 fold in f
 TG: ↑ 1.7 fold in m and 2.5 fold in f
 Urea nitrogen: ↑ 14% males only
 AST: ↓ 17% m and 23% f
 ALP: ↓ 20% m and 26% f
 Calcium: ↑ 4.7% in both sexes

The only remaining changes at recovery d29 were increase in total cholesterol in males (18% over control), increase in total proteins in females (6% over control), urea nitrogen increased 16% over control in f, and AST decreased in f 11% less than control. All parameters were comparable to control by end of 2nd recovery period. Mean T3 values were significantly ($p \leq 0.01$) less than the controls in dosed females (38% less) and males (11% less) and TSH values were 2.8 fold more than the controls in females only. **All the above changes were small and/or within historical range.**

Gross Necropsy: enlarged and dark discolored livers was seen in both sexes at end of dosing (almost all rats) as well as enlarged adrenals (3m & 4f). None of these findings were seen by end of recovery periods.

Organ wts: at end of dosing period, mean absolute and relative wts of the liver in both sexes were significantly increased (1.5-1.8x) over the corresponding control values. Mean absolute and relative adrenal wts in both sexes were also increased significantly over the controls (absol 18&14% m&f; rel. 28&23% m&f over the controls respectively). Mean relative wt of kidneys in males and females was increased 11&4% respectively. over the controls; no effect on absolute wt. Mean relative wt of testes was increased 8.8% over the control. At end of 4wk recovery, the only changes were a significant increase in absolute (24%) and relative (25%) wts of the spleen in males, 19% increase in relative wt of pituitary in males, a significant increase in mean absolute (14%) and relative (12%) wts of the liver only in females, and a significant 8% increase in mean absolute wt of ventral prostate. **There were no findings in any organ/tissue by end of recovery wk13.**

Histopath:

End of dosing:

Liver: centrilobular hepatocyte hypertrophy in all m&f; none in controls.
 Intracytoplasmic inclusion bodies in hepatocytes in 1f.
 Adrenals: increase in vacuolization of cortical cells (mild-moderate) in all m&f; none in controls. The vacuolization was characterized by an intracytoplasmic increase in fine vacuoles in zona fasciculata though there were many large vacuoles in 2males
 Ovaries: interstitial cell vacuolization in 6/10f; none in control.

End of 4wk recovery:

Adrenal vacuolization in 3/10m and 2/10f : none in controls.

End of 13wk recovery:

No findings.

Summary and Conclusion:

Oral gavage administration of TAK-375 to rats at 600mg/kg/d for 1month did not cause death and was well tolerated. Clinical signs were limited to salivation and chromaturia. Drug related findings included very small but significant decrease in mean wt gain in males, food intake in females, decrease in T3 in both sexes and increase in TSH in females only, and increases in wts of the liver and adrenals both accompanied by histopathology. All findings were reversed by wk13 of recovery, few observations remained at end of 4wk recovery. The 600mg/kg/d dose is not an MTD and may be considered a LOEL since the drug related findings may be extensions of the pharmacology of a melatonin agonist.

Study title: Four-Week Oral Gavage Range-Finding Toxicity Study of TAK-375 in Rats**Key study findings:**

Note: This study was not reviewed formally but was examined for dosing and toxicity information used for guidance of the design of subsequent dose range-finding studies for the carcinogenicity study in the rat.

*Note: This study utilized the **Wistar** rat strain whereas the carcinogenicity study utilized a **Sprague-Dawley** strain.*

- Vehicle (0.5 w/v% MC) or TAK-375 at doses of 40, 150, 600 or 1500 mg/kg/day were administered by oral gavage to Wistar rats for 4 weeks followed by a 13-week recovery in satellite animal groups.
- Mortality was observed in 2/5 females at the 1500 mg/kg/day dose within the first two study days.
- Clinical signs included licking at all TAK-375 doses and salivation with dosing $\geq$ 150 mg/kg/day. Decreased locomotor activity and prone position was noted in males and females on the first study day only with dose levels of $\geq$ 600 mg/kg/day.
- Decreased body weight gain was observed in males at a dose of 1500 mg/kg/day. No effect on body weight in females at any dose level.
- Food consumption was reduced in both males and females at a dose of 1500 mg/kg/day.
- Hematologic changes occurred with dose levels of $\geq$ 600 mg/kg/day in males and at 1500 mg/kg/day in females and involved increased platelets and increased reticulocytes (females) with reductions in hemoglobin (females only) and hematocrit.

- Clinical chemistry changes were observed with dose levels of ≥ 600 mg/kg/day and involved increased total protein (males) and decreased A/G ratio (females) at a dose of 600 mg/kg/day while at the 1500 mg/kg/day increases in total protein, total globulin, decreased A/G ratio, urea nitrogen (males) and creatinine (males) was observed.
- Assessment of thyroid hormones indicated a reduction in T3 and elevation in TSH in plasma with dose levels ≥ 600 mg/kg/day while a significant reduction in T4 was only found at 1500 mg/kg/day.
- Assessment of hepatic metabolizing enzyme activity indicated TAK-375 at doses of ≥ 150 mg/kg/day was associated with an increase in aminopyrine-N-demethylase activity in liver samples of both sexes while aniline hydroxylase was observed to be elevated in activity only in females and only at the 150 and 600 mg/kg/day dose levels, therefore this effect on aniline hydroxylase is likely to be artifactual as it is not dose related and only observed in one sex while the increase in aminopyrine-N-demethylase is considered real and toxicologically significant.
- Necropsy exam revealed effects on the liver and thyroid with doses of ≥ 600 mg/kg/day consisting of enlargement and dark discoloration and dark discoloration, respectively, while enlargement of the adrenal gland was observed only at a dose of 1500 mg/kg/day. Organ weights were reflective of the necropsy exam and indicated the liver and adrenal glands were significantly increased in weight (females) with a dose of 150 mg/kg/day while this was seen in both genders, as well as an increase in thyroid weight, with a dose of ≥ 600 mg/kg/day. Additionally, at TAK-375 doses ≥ 150 mg/kg/day, the ovaries were also observed to be increased in weight.
- Histopathology revealed TAK-375-related effects with doses ≥ 150 mg/kg/day consisting of **Liver:** Centrilobular hepatocellular hypertrophy, with periportal vacuolization of hepatocytes at a dose of 1500 mg/kg/day; **Thyroid:** Follicular cell hypertrophy with doses ≥ 150 mg/kg/day; **Adrenal:** Cortical cell vacuolization with dose levels ≥ 150 mg/kg/day; and, **Ovary:** Interstitial cell vacuolization with dose levels ≥ 150 mg/kg/day.
- The NOAEL identified by the Sponsor, which appears appropriate to this reviewer on inspection, is **40 mg/kg/day**. The toxicokinetic assessment conducted is reproduced from the Sponsor's table below.

Dosage (mg/kg/day)	0	40	150	600	1500
Plasma drug concentration ^b (M:F)					
Tmax (h)	1st	0.3 : 0.3	0.3 : 0.3	0.3 : 1.3	0.3 : 2.1
	29th	0.5 : 0.3	0.3 : 0.3	0.3 : 0.3	3.1 : 1.2
Cmax ($\mu\text{g/mL}$)	1st	2.65 : 3.06	5.01 : 8.59	8.92 : 29.72	10.10 : 33.56
	29th	1.39 : 2.67	1.81 : 6.24	6.86 : 10.08	7.80 : 11.64
AUC _{0-24h} ($\mu\text{g}\cdot\text{h/mL}$)	1st	1.9 : 4.1	19.7 : 48.0	51.0 : 218.8	132.7 : 424.4
	29th	2.0 : 3.1	5.7 : 8.8	19.5 : 27.1	85.5 : 68.6

Study no.: M-11-00608

Volume #, and page #: Module 4.2.3.4.1 Long Term Studies

Conducting laboratory and location: ☐ Drug Safety Research Laboratories,
Takeda Chemical Industries, Ltd. Yamaguchi, 743-8502, Japan

Date of study initiation: August 12, 1998

GLP compliance: No

QA report: yes () no (X)

Drug, lot #, and % purity: TAK-375, Lot # M375-006, Purity [] Stability and homogeneity of test article in suspension confirmed in Appendix report

Methods

Doses: 0 (0.5 w/v% methylcellulose solution), or TAK-375: 40, 150, 600 or 1500 mg/kg/day in 0.5 w/v% MC for 4 weeks followed by 13 week recovery

Species/strain: Jcl: Wistar rats. Note: Sprague-Dawley used for carcinogenicity study

Number/sex/group or time point (main study): 5 rats/sex/group

Route, formulation, volume, and infusion rate: Oral gavage in 0.5 w/v% MC suspension, 5 mL/kg/day

Satellite groups used for toxicokinetics or recovery: 4 rats/sex/group for recovery assessment

Age: 6 Weeks at initiation of dosing

Weight: Not specified

Sampling times: Toxicokinetic assessment done with 3 animals/sex/group for the following time-points: pre-dose (29th Dose sample only), 0.25, 0.5, 1, 3, 6, and 24 hr after drug administration on the 1st (SD1) and last day of the study (SD29)

Unique study design or methodology (if any): No unique aspects of the Study except the use of Wistar strain rather than the Sprague-Dawley strain. Sponsor did assess thyroid hormones. First day of drug administration is SD1.

Results

This study was not reviewed formally but was examined for dosing and toxicity information used for guidance of the design of subsequent dose range-finding studies for the carcinogenicity study in the rat. Please see Key Findings.

Appears This Way
On Original

Study title: 13-Week Oral Gavage Range-Finding Toxicity Study of TAK-375 in Rats

Note: This study was reviewed by Aisar Atrakchi, Ph.D. in Serial Submission #013

2. 13wk oral gavage range finding tox study of TAK-375 in rats/M-11-171/study# 2934/ca

Conducting lab: Takeda, Japan

GLP: Yes/Japan

QA report: Yes

Initiation/Termination dates: Feb 1999/May 1999

Drug lot#/purity: M375-010, M375-012, M375-013, []

Strain/# per sex per dose: **Wistar** rats; 10/sex/dose; additional 4/sex/dose for TK.

Doses & vehicle: **40, 150, 600, 1200mg/kg/d** and the control group received the vehicle: 0.5%w/v methylcellulose. Administration volume: 5ml/kg.

Route/Duration: daily oral gavage for 13wks

B.wt/age at initiation: 6wks/163-182g males and 126-144g females

Parameters assessed:

Mortality & Clinical Signs: 1x predose and during treatment 2x daily before dose at 0hr and 2hr postdose. Detailed exam was done once predose and once weekly during dosing.

B.wt: 2x predose, 1st day of dosing before dose and once weekly thereafter.

Food intake: once weekly.

Hematology & Blood Chemistry: standard assessment for both parameters was done at end of dosing. Animals were fasted overnight and blood was collected from the abdominal aorta.

T3, T4, and TSH were also measured.

Liver Enzyme Activity: at necropsy, pieces of livers were removed from 5/sex/dose and processed to determine aminopyrine-N-demethylase and aniline hydroxylase activities.

Gross Morphology and Organ wts: organs/tissues were examined visually and the absolute and relative wts of the following organs were determined at necropsy: brain, thymus, heart, lungs, liver, spleen, kidneys, testes/ovaries, pituitary, adrenals, and ventral prostate.

Histopathology: a number of tissues/organs from all groups were fixed in 10% buffered formalin. All tissues except oviducts, nose, extraorbital lacrimal glands, and Zymbal's glands, were embedded in paraffin. All embedded tissues from control and 1200mg/kg group and livers, kidneys, pituitary, thyroids, adrenals, thymus, and ovaries from the 40, 150, and 600mg/kg groups were processed and stained with H&E for LM.

TK: blood was collected from the jugular vein of each satellite animal after the 1st dose and after the final dose (87th dose) at 0.25, 0.5, 1, 3, 6, and 24hr postdose.

Results:

Mortality: 1f dosed 1200mg/kg was found dead on d3 of dosing. cause was contributed to the drug as well as stress related to blood sampling. *This female was #129 in the satellite group dosed 1200mg/kg/d, no raw data was provided for this female therefore, the reviewer could not accurately describe the cause of death in this animal. Note that this is perhaps because no observations or histopath exam were done on the satellite group.*

Clinical signs: motor activity was reduced in 1f dosed 600mg/kg during 1st wk of dosing and in 1f dosed 1200mg/kg during wk5, salivation noted in both sexes dosed 1200mg/kg at ≥wk4 and in 1m dosed 600mg/kg during wk8, chromaturia seen in both sexes dosed 1200mg/kg at ≥wk8, distended abdomen was seen in 2-3f dosed 1200mg/kg on wks11, 12, and 13, and licking was seen in 1/sex dosed 1200mg/kg. These signs were not present by the next day prior to dosing.

B.wt: mean wt gain was reduced (p≤0.05 or 0.01) in females throughout the dosing period starting from wk3 in 150mg/kg (4.6%) and 600mg/kg (4.6%), onward in all female drug groups; the decrease in the top dose of 1200mg/kg/d started on wk5. In males, wt gain was reduced in all drug groups from wk7 onward and in HD from wk4 onward. Table below presents the % reduction in gain relative to corresponding controls in all drug groups from wk5-13 at which period the change reached statistical significance (the values represent the % reduction in wt for 2 or all 4 drug groups):

Weeks	% Reduction in wt Gain	
	Males	Females
5	6-10	5-8
6	6-12	5-8.5
7	6.6-13	5-10
8	7.7-14	5-11
9	8-15	5-10
10	7-16	6-11
11	7.6-16.6	5-10
12	7-17	7-11
13	7-17	8-11

Note that the gain reduction in males dosed 1200mg/kg ranged between 6-17% between wks5-13 of dosing and the gain reduction in females dosed 600mg/kg ranged between 8-11% between wks5-13. Change in mean weight gain in females dosed 1200mg/kg did not reach statistical significance and during some wks was comparable to the controls. This latter result could be related to increased food intake in this female group during some of these periods. In males dosed 600mg/kg, wt gain was reduced between 7-8% during wks 8-13 relative to the control.

Food Intake: there was no drug effect on intake except as noted under B.wt above where intake in females dosed 1200mg/kg was slightly but significantly increased ($p \leq 0.05$) during some periods.

Hematology: there were small but statistically significant changes in some parameters in both sexes usually in 600 & 1200mg/kg dose groups they included

Platelets: ↑ dose dependent 12&26% in m and 23% in HDf
 Retics ↑ dose dependent 15&31% in m and 28% in HDf
 Lymphos ↑ 7% in HDm and 7% in 600&1200mg/kg f

Clinical Chemistry: the following reached statistical significance ($p \leq 0.05$ or 0.01):

		600&1200mg/kg/d % change from control
Total proteins	m	↑6.5&7
	f	↑7.7&17
Total Choles	m	↑37&80
	f	↑48&60
Calcium	m	↑8&11.6
	f	↑9&16.6
TSH	m	↑3.3&5 times control
	f	↑7.6&8 times control
T3	f	↓19&21; no change in males
A/G ratio	m	↓12 in HD; no change in 600mg/kg
	f	↓12&24

Hepatic Enzyme Activity: mean absolute and relative enzyme activity was increased significantly but not dose dependently only in females: amino-N-demethylase (relative to liver: 85, 120, 66% in 150, 600, & 1200mg/kg respectively) and aniline hydroxylase activity (62&129% in 150&600mg/kg respectively).

Gross morphology: the following organs were enlarged and discolored: liver, adrenals, kidneys, thyroids, and ovaries, thymus was small in 3/10m dosed 600mg/kg and in 5/sex dosed 1200mg/kg.

Organ wt: mean absolute and relative wt of the liver was significantly increased in males (relative: 1.7&2.4x control in 600&1200mg/kg respectively), and females (relative: 2&3x control in 600&1200mg/kg respectively). Table below from sponsor presents changes in relative wts:

Organ weights (body weight ratio) - Group mean values, (mean±S.D.)

Group	Compound	Dose (mg/kg/day)	1	2	3	4	5
			Control	TAK-375 40	TAK-375 150	TAK-375 600	TAK-375 1200
Group Number of animals	Body weight (g)	Heart	Lungs	Liver	Kidneys	Spleen	Pituitary gland (x10 ⁻³)
1M 10	420± 22	0.27±0.01	0.27±0.01	2.75± 0.08	0.61±0.04	0.17±0.01	2.8± 0.1
2M 10	389± 19*	0.28±0.01	0.28±0.01	2.82± 0.08	0.61±0.03	0.16±0.01	2.3± 0.1
3M 10	383± 20**	0.29±0.01**	0.29±0.02**	3.16± 0.13*	0.64±0.04	0.18±0.02	2.5± 0.1
4M 10	387± 27**	0.31±0.02**	0.29±0.02**	4.68± 0.16**	0.72±0.03**	0.19±0.02	2.8± 0.1
5M 10	339± 22**	0.33±0.01**	0.31±0.02**	6.57± 0.29**	0.81±0.05**	0.19±0.03	2.9± 0.1
Group Number of animals	Thyroids (x10 ⁻³)	Adrenal glands (x10 ⁻³)	Testes (x10 ⁻³)	Brain (x10 ⁻³)	Ventral prostate (x10 ⁻³)	Thymus (x10 ⁻³)	Carcass (x10 ⁻³)
1M 10	4.5± 0.7	13.7± 1.1	0.78±0.03	0.48±0.03	81± 26	46± 9	80± 1
2M 10	5.1± 0.7	13.6± 1.2	0.82±0.05	0.50±0.02	64± 15	64± 14**	81± 1
3M 10	5.1± 0.6	14.3± 1.3	0.85±0.06**	0.51±0.03*	70± 15	64± 12**	79± 1
4M 10	6.4± 0.8**	16.8± 1.9*	0.84±0.03*	0.50±0.03	69± 18	46± 16	77± 1
5M 10	7.6± 0.3**	28.5± 6.5**	0.98±0.05**	0.56±0.04**	73± 18	41± 7	73± 1
Group Number of animals	Body weight (g)	Heart	Lungs	Liver	Kidneys	Spleen	Pituitary gland (x10 ⁻³)
1F 10	254± 6	0.32±0.02	0.35±0.02	2.42± 0.11	0.64±0.03	0.21±0.01	3.0± 0.1
2F 10	242± 11*	0.33±0.02	0.35±0.02	2.49± 0.09	0.65±0.04	0.22±0.02	4.1± 0.1
3F 10	232± 13**	0.34±0.02	0.37±0.02*	3.11± 0.14*	0.70±0.05**	0.22±0.01	4.3± 0.1
4F 10	223± 9**	0.36±0.01**	0.39±0.01**	5.13± 0.24**	0.74±0.04**	0.24±0.02**	4.6± 0.1
5F 10	244± 12	0.38±0.02**	0.38±0.02**	8.10± 0.70**	0.82±0.05**	0.23±0.01**	4.2± 0.1
Group Number of animals	Thyroids (x10 ⁻³)	Adrenal glands (x10 ⁻³)	Ovaries (x10 ⁻³)	Brain (x10 ⁻³)	Thymus (x10 ⁻³)	Carcass ?	
1F 10	5.9± 0.9	26.2± 3.0	36.8± 4.6	0.72±0.02	79± 16	81± 1	
2F 10	6.6± 0.8	25.8± 3.4	38.5± 5.1	0.78±0.04*	87± 13	80± 1	
3F 10	7.0± 1.2	28.7± 2.4	39.3± 6.2	0.80±0.05**	87± 14	80± 1	
4F 10	8.2± 1.0**	41.1± 5.4**	55.6± 16.8**	0.82±0.04**	89± 9	76± 1**	
5F 10	10.3± 1.3**	55.2± 10.0**	76.9± 22.8**	0.74±0.05	59± 16**	69± 2**	

Significantly different from group 1 (*:p≤0.05 or **:p≤0.01)
The values are expressed as a percentage of body weight.

Histopathology:

- Liver:**
- centrilobular hepatocyte hypertrophy 2/10m dosed 40mg/kg and in both sexes dosed ≥150mg/kg.
 - intracytoplasmic vacuolization of periportal hepatocytes in 6/10m and 7/10f dosed 1200mg/kg.
 - Diffuse hepatocyte pigmentation in 5/10m and 7/10f dosed 600mg/kg and 9/10m and all f dosed 1200mg/kg.
 - Sinusoidal cell pigmentation was seen in 1m and 5f dosed 1200mg/kg.

- Thyroids:**
- follicular cell hypertrophy in 3m dosed 40mg/kg. 9m and 4f dosed 150mg/kg. and all rats dosed 600&1200mg/kg.

Histopath (Cont.):

Adrenals: - vacuolization of cortical cells in 5m and 3f dosed 40mg/kg. all m and 9f dosed 150mg/kg and in all rats dosed 600&1200mg/kg.

Ovaries: - vacuolization of interstitial cells in 7f dosed 40mg/kg and all f dosed ≥150mg/kg.

Kidneys: - hyaline casts were increased in incidence or severity in m dosed 600&1200mg/kg and f dosed 1200mg/kg.
 - pigmentation of renal tubules was increased in incidence in m dosed 1200mg/kg and f dosed 600&1200mg/kg.

Thymus: - atrophy in 7m dosed 600mg/kg and 7m and 2f dosed 1200mg/kg (2m control, none in f controls).

Table below from sponsor summarizes the above findings:

Test article	Control*	TAK-375				
Dosage level (mg/kg/day)	0	40	150	600	1200	
Dosage volume (mL/kg)	5	5	5	5	5	
No. of animals ¹⁾ (M:F)	10:10	10:10	10:10	10:10	10:10	
Histopathology	Liver: (M2)	Hypertrophy of hepatocytes (M10:F10) (M10:F10)		Vacuolization of hepatocytes# (M6:F7) (M10:F10)		
	Thyroids: (M3)	Hypertrophy of follicular cells# (M9:F4) (M10:F10)		(M10:F10)		
	Adrenals: (M5:F3)	Vacuolization of cortical cells# (M10:F9) (M10:F10)		(M10:F10)		
	Ovary: (F7)	Vacuolization of interstitial cells# (F10) (F10)		(F10)		
	Kidney:	Basophilic renal tubules# (M1)	(M7)	(M10:F3)		
		Cast# Pigmentation of renal tubules# (M5) (F6)	(M5)	(M6:F2) (M6:F7)		
Thymus:	Atrophy#	(M7)	(M7:F2)			

*: 0.5 w/v% methyl cellulose solution, - : No treatment-related effects
 #: Toxicologically significant changes, M: Male, F: Female.

TK: Mean C_{max} of parent increased less than proportional to increase in dose following single (1st) and repeated dosing. Exposure also increased non-linearly with dose but inconsistently. Both parameters were higher in females than males and values decreased after repeat dosing. The major metabolite was M-I followed in decreasing order by: M-III, M-II, and M-IV (table below from sponsor):

Test article	Control*	TAK-375			
Dosage level (mg/kg/day)	0	40	150	600	1200
Dosage volume (mL/kg)	5	5	5	5	5
No. of animals ¹⁾ (M:F)	10:10	10:10	10:10	10:10	10:10
Plasma conc. ⁴⁾ (M:F)					
C _{max} (ng/mL)	1st TAK-375	1737 : 6217	5363 : 15283	11720 : 22677	16210 : 44303
	87th TAK-375	673 : 1510	1932 : 7958	3847 : 9866	7860 : 14778
	M-I	938 : 721	2048 : 2378	5569 : 5133	10067 : 7326
	M-II	131 : 733	268 : 1802	355 : 1205	553 : 1497
	M-III	407 : 749	1016 : 3124	2242 : 5402	4779 : 9638
	M-IV	35 : 100	58 : 285	85 : 277	168 : 421
AUC ₀₋₂₄ (ng·h/mL)	1st TAK-375	2423 : 7510	31200 : 62743	84293 : 165297	146173 : 738070
	87th TAK-375	1154 : 2774	7724 : 12353	14251 : 41611	41242 : 175373
	M-I	2096 : 1797	9992 : 6205	23408 : 24728	64548 : 66321
	M-II	390 : 2890	1689 : 7248	1730 : 6823	3604 : 14215
	M-III	1304 : 3128	6229 : 12088	14619 : 41420	43306 : 137406
	M-IV	222 : 1149	551 : 1959	751 : 2630	1784 : 5643

*: 0.5 w/v% methyl cellulose solution, - : No treatment-related effects

1): Additional 4 animals/sex/dose were used as satellite groups for toxicokinetics.

4): Mean values of 3 animals

Summary and Conclusion:

Oral gavage administration of TAK 375 to male and female rats for 13wk at 40, 150, 600, and 1200mg/kg/d caused death in 1f dosed 1200mg/kg after 3 days of dosing. Clinical signs were limited to salivation and chromaturia in high dose. Mean wt gain was significantly reduced in all drug groups and in HDm the decrease ranged between 6-17% and in females dosed 600mg/kg was 8-11% respectively. Mean food intake was not affected except for an increase in HDf that may explain the unaffected mean wt in this group. There were some changes in hematology parameters seen in both sexes, reached statistical significance, and were dose dependent: increase in platelet, retics, and lymphos in 600&1200mg/kg (7-31% change from control). At 600&1200mg/kg/d mean total proteins, cholesterol, and calcium were significantly increased in males and females relative to the control (6.5-80%). Mean TSH was increased in both sexes dosed 600&1200mg/kg between 3-8x control whereas T3 was decreased only in f at these doses (19&21%). Mean absolute and relative wt of the liver was significantly increased which was accompanied by histopathology and increase in enzyme induction. Weights of kidneys, adrenals, thyroids and ovaries were also enlarged and discolored.

Histopathology included liver centrilobular hepatocyte hypertrophy in both sexes dosed ≥ 150 mg/kg, intracytoplasmic vacuolization of periportal hepatocytes in 6/10m and 7/10f dosed 1200mg/kg, diffuse hepatocyte pigmentation in 5/10m and 7/10f dosed 600mg/kg and 9/10m and all f dosed 1200mg/kg, sinusoidal cell pigmentation in 1m and 5f dosed 1200mg/kg, thyroid follicular cell hypertrophy in 3m dosed 40mg/kg, 9m and 4f dosed 150mg/kg, and all rats dosed 600&1200mg/kg, adrenal vacuolization of cortical cells in 5m and 3f dosed 40mg/kg, all m and 9f dosed 150mg/kg and in all rats dosed 600&1200mg/kg, ovarian vacuolization of interstitial cells in 7f dosed 40mg/kg and all f dosed ≥ 150 mg/kg, kidney hyaline casts were increased in incidence or severity in m dosed 600&1200mg/kg and f dosed 1200mg/kg, pigmentation of renal tubules was increased in incidence in m dosed 1200mg/kg and f dosed 600&1200mg/kg, and thymus atrophy in 7m dosed 600mg/kg and 7m and 2f dosed 1200mg/kg. Mean C_{max} of parent increased less than proportional to increase in dose following 1st and repeated dosing. Exposure also increased non-linearly with dose but inconsistently. Both parameters were higher in females than males and values decreased after repeat dosing. The major metabolite was M-I followed in decreasing order by: M-III, M-II, and M-IV. Based on these results the MTD is <1200 mg/kg/d, NOAEL is 40mg/kg and the LOEL is 150mg/kg/d. However, it should be noted that the histopathology findings are likely to be extensions of the pharmacology of the drug (melatonin agonist effects), and therefore 600mg/kg/d may be considered the NOEL.

Study title: Twenty-Six Week Oral Gavage Toxicity Study of TAK-375 in Rats

Key study findings:

- Salivation observed in both males and females administered oral doses ≥ 40 mg/kg/day. This finding was reduced in incidence and severity as the study progressed but was still present.
- Mild hematologic changes in males and females at the 150 mg/kg/day dose level only. Female rats had a decrease in erythrocytes (-6%) while both males and females had decreased hematocrit (-4%, m; -6%, f) and hemoglobin (-5%, m; -8% f). Additionally, females at 150 mg/kg/day were also observed to have a significant increase in platelets (+14%) relative to female control animals.
- Mild clinical chemistry changes were noted only in the 150 mg/kg/day groups of both male and female rats. Statistically significant changes included an increase in total protein in both sexes (+6%, m; +9%, f) with albumin being increased in high dose males (+6%) while circulating A/G levels were lower in females (-10%) versus control animals. Female rats at the 150 mg/kg/day had an increase in their total cholesterol levels (+69%) at the end of the study period relative to controls.
- Assessment of circulating T3 and TSH levels indicated that females at 150 mg/kg/day had a reduction in T3 levels by approximately 14% while TSH increased nearly 123% when compared with control animals. A trend towards these findings was

not found in female dose-groups below 150 mg/kg/day and was not observed in male animals at any dose level.

- Post-mortem examination revealed dark and enlarged livers in females at 150 mg/kg/day. This correlated with increased liver weight at 150 mg/kg/day with females more greatly affected than males (+14%, m; +49% females). Other organ weights which were observed to be altered as a result of treatment were: increased thyroid weight in males with dosing $\geq$ 40 mg/kg/day (+19%, 40 mg/kg/day; +26%, 150 mg/kg/day) while females demonstrated this increased thyroid weight only at the 150 mg/kg/day dose level (+16%) when compared with control animals. Evidence of increased kidney weight (11%), spleen weight (+16%) and adrenal gland weight (+15%) were also observed in high dose females only.
- Microscopic analysis of tissues revealed evidence of TAK-375-related changes in the liver, adrenal gland, thyroid gland, spleen of male and female rats as well as in the ovaries of females. Principal findings in liver consisted of centrilobular hepatocellular hypertrophy with dosing $\geq$ 40 mg/kg/day such that nearly all animals exhibited this finding at the 150 mg/kg/day dose level and which was characterized as being minimal-mild in severity at this highest dose; a finding which correlates with the increased liver weight. Adrenal cortical vacuolization, characterized as minimal in severity was observed in males at the 150 mg/kg/day dose and in a minority of female rats with dosing $\geq$ 40 mg/kg/day. Evidence of thyroid follicular cell hypertrophy was apparent in males and females at the 150 mg/kg/day dose level. One male each at the 10 and 40 mg/kg/day dose level also showed this finding. Virtually all animals demonstrated extramedullary hematopoiesis and pigmentation of the spleen at all dose levels including vehicle control though the severity was slightly worse in the females given 150 mg/kg/day. In females at the 150 mg/kg/day dose, minimal to mild intracytoplasmic vacuolization was found in the majority of animals but was not observed with lower dose groups.
- TAK-375 exposure increased generally in a dose-related manner with a mild accumulation over the 26-week study period with dosing $\leq$ 40 mg/kg/day though a reduction in exposure during the study was observed in the 150 mg/kg/day treatment group, suggestive of increased drug metabolism which is supported by liver findings. Females generally had higher systemic exposures than males to TAK-375 and all metabolites except M-I which was observed to be higher in males than females with dosing of $\geq$ 40 mg/kg/day measured in the terminal sample in the study (the only samples which were assessed for metabolite concentration).
- The principal toxicologic findings of this 26-week study of TAK-375 in the rat include:
 1. a **mild anemia** suggested by the hematologic changes, increased spleen weight and increased degree of hematopoietic cell proliferation;
 2. **increased thyroid weight and thyroid activity changes** likely due to increased metabolism and excretion of T3 (which was observed to be reduced

Group	Test article	Dosage (mg/kg/day)	Volume (mL/kg)	Conc. (w/v%)	No. of animals (Animal No.)	
					Male	Female
1	control	0	10	0	15 (1-15)	15 (96-110)
2	TAK-375	2.5	10	0.025	15 (16-30)	15 (111-125)
3	TAK-375	10	10	0.1	15 (31-45)	15 (126-140)
4	TAK-375	40	10	0.4	15 (46-60)	15 (141-155)
5	TAK-375	150	10	1.5	15 (61-75)	15 (156-170)
6*	TAK-375	2.5	10	0.025	5 (76-80)	5 (171-175)
7*	TAK-375	10	10	0.1	5 (81-85)	5 (176-180)
8*	TAK-375	40	10	0.4	5 (86-90)	5 (181-185)
9*	TAK-375	150	10	1.5	5 (91-95)	5 (186-190)

*: These were the satellite groups for determination of plasma drug concentrations. The data on body weight and clinical signs for these groups were not used for evaluation of toxicity.

Results

Mortality: Assessed with daily morning observations of clinical signs before dosing. No animals died during the 26-week study.

Clinical signs: Observed daily prior to study initiation and then twice daily in the morning (pre-dose and 0 – 2 hr post-dose) during the treatment period. Animals in the main study groups underwent extensive clinical examination once during the pre-treatment period and then once a week during the 26 week study. These extensive clinical examinations were carried out both prior to dosing and 0 – 2 hr post-dose.

The primary clinical sign attributable to test-article treatment was salivation which was noted in a significant number of male and female rats treated at the 40mg/kg/day level and in all animals at the 150 mg/kg/day dose level. Salivation occurred immediately after dosing and was not seen prior to dosing at any time. In addition, the incidence was reduced during the course of the study and in nearly all 40 mg/kg/day group rats and the majority of 150 mg/kg/day group rats salivation disappeared with repeated dosing over time. Interestingly, salivation was not observed at any dose until the week 3 of dosing. This observation is not mentioned by the Sponsor nor is an explanation therefore given.

Body weights: Body weight was taken twice during the pre-treatment period and then once prior to dosing on Day 0 and then once weekly during the treatment period and prior to necropsy.

Body weight was not observed to be altered by treatment with TAK-375 at any dose level.

Food consumption: Determined once during the pre-treatment period for each animal and then once weekly for animals in groups 1-5 for the first 14 weeks of the study followed by once every 4 weeks until the conclusion of the study. Several animals in each treated group (including control) were infrequently excluded from analysis due to “spillage” of diet.

High dose females (150 mg/kg/day) assessed at week 26 had significantly increased food consumption relative to controls. Otherwise, no differences were found.

Ophthalmoscopy: Examination was performed during the pre-treatment period and then 5 animals were examined from each sex in main study groups during weeks 13 and 26. Ocular

media and fundus were examined in both eyes of the animal using an ophthalmoscope with an [] lens.

Although several ophthalmologic findings were observed, none appeared to be test-article related as they were found in control groups and/or did not demonstrate dose-dependent increases in incidence or severity.

EKG: Not examined.

Hematology: All animals in the main study groups were fasted for approximately 20 hr prior to blood collection from the abdominal aorta under ether anesthesia during necropsy. An [] hematology analyzer was used for analysis while the differential leukocyte count was obtained by microscopy after May-Giemsa staining. All but one male in the control group (sample coagulated) were examined in this manner. The following parameters were assessed:

- erythrocyte count	method)
- leukocyte count	method)
- platelet count	method)
- hematocrit	method)
- hemoglobin concentration	method)
- mean corpuscular volume (MCV, calculated)	
- mean corpuscular hemoglobin (MCH, calculated)	
- mean corpuscular hemoglobin concentration (MCHC, calculated)	
- reticulocyte count (flow cytometry [] method)	
- differential leukocyte count	

Apparent treatment-related alterations in hematologic parameters were observed in both males and females but only at the highest dose tested. Male rats treated with 150 mg/kg/day of TAK-375 at sacrifice demonstrated a mild but statistically significant reduction in hematocrit (-4%) and hemoglobin (-5%) versus control animals with a non-significant decrease in erythrocytes (-3%). Female rats in the 150 mg/kg/day group showed a statistically significant, though mild, reduction in erythrocytes (-6%), hematocrit (-6%), hemoglobin (-8%), mean cell hemoglobin concentration (-1%, significant also), and an increase in plasma platelet concentration (+14%) relative to female control animals.

Clinical chemistry: The following parameters were obtained from the blood or plasma of all main study animals (except the single control male rat) prior to necropsy on the last day of dosing following ~ 20 hr fasting and values obtained using automated analyzers.

- total protein (method)	- sodium (method)
- albumin (method)	- potassium (method)
- A/G ratio	- chloride (method)

- glucose (method)
- total cholesterol (method)
- triglyceride (method)
- urea nitrogen (method)
- creatinine (method of Jaffé)
- total bilirubin (method)
- aspartate aminotransferase (AST: method)
- alanine aminotransferase (ALT: method)
- lactate dehydrogenase (LDH: method)
- alkaline phosphatase (ALP: method)
- creatine kinase (CK: method)
- calcium (method)
- inorganic phosphorus (method)

In addition, 8 animals from each main study group were sacrificed in the afternoon and blood collected for analysis of T3 and T4 levels (EIA method) and TSH levels (CLIA method).

Male and female rats at the 150 mg/kg/day dose level demonstrated significant increases in total protein (+6% in males, +9% in females) with males showing an increase in albumin (+6%) and females a decreased A/G ratio (-10%). Females also demonstrated significantly increased total cholesterol at the 150 mg/kg/day dose level, +69% (164 ± 32 mg/dL) above control animals and an increase in blood concentrations of calcium was observed in both sexes but this was well within the range of historical control values as described by Sponsor. Females demonstrated a significant reduction in T3 levels (-14% vs. controls) and an increase in TSH (+123%) at the 150 mg/kg/day dose level, a finding which was not observed in males. All other alterations of statistical significance involving blood chemistry parameters were not dose-related and only seen in one sex as well as being within normal historical background ranges for the strain of rat. No statistically significant changes were observed with dosing of 40 mg/kg/day or below.

Urinalysis: 5 animals/sex/group were analyzed for water intake and urine output over 24 hr during weeks 13 and 26 of the treatment period. One animal was excluded from the 10 mg/kg group during week 13 due to bottle water leakage.

No treatment-related effects were observed on either water intake or urine output.

Urinalysis consisted of 4-hr urine samples from 5 animals/sex/group (same animals) during a brief fast. The following urinalysis parameters were measured:

- pH
- protein
- glucose
- ketones
- occult blood
- urobilinogen

In addition, microscopic examination of urine sediment was fixed and stained to examine for potential presence of the following:

- casts
- epithelial cells
- leukocytes
- erythrocytes

No treatment-related alterations in urinalysis parameters were observed with analysis of urine or urine sediment.

Gross pathology:

Animals in groups 1-5 (main study animals) were fasted overnight and weighed before sacrifice by ether anesthesia. During necropsy individual visceral organs and tissues were examined macroscopically.

The only apparent macroscopic finding that appeared to be treatment-related was a dark liver in 8/15 females and enlarged liver in 4/15 females at the 150 mg/kg/day dose level. These findings were absent in male rats. A small increase in the incidence of small thymus was found in males as dose increased with 0 controls, 1 male at 2.5 mg/kg/day and 10 mg/kg/day; 2 males at 40 and 150 mg/kg/day having this finding reported out of 15 animals at each dose level.

Organ weights (specify organs weighed if not in histopath table):

The following organs were weighed and calculated also as a percentage of body weight:

Brain	Spleen	Ventral Prostate
Heart	Pituitary	Ovaries
Lungs	Adrenals	Thyroids
Liver	Thymus	
Kidneys	Testes	

Body weight was not observed to be significantly different between treatment groups in this study at termination. Consequently, a change in the absolute weight of selected organs appears sufficient to detect test-article-related effects. In both males and females at 150 mg/kg/day, a significant increase in liver weight was found (+12%, m; +43%, f) which was not apparent at lower dose levels. Males and female rats also had increased thyroid weight with males in both 40 mg/kg/day (+19%) and 150 mg/kg/day (26%) being significantly increased compared with controls while in females only the 150 mg/kg/day group value was significantly different from control (+16%). Female rats at 150 mg/kg/day additionally appeared to have an increase in kidney weight (+11%), spleen weight (+16%) and adrenal gland weight (+15%) with lower dose groups showing this trend relative to female controls.

Histopathology: Adequate Battery: yes (X), no ()—explain

Peer review: yes (), no (X)

The organs and tissues listed below from all main study animals were fixed according to accepted guidelines. All tissues listed below which do not have an asterisk were embedded in paraffin. All tissues from the control group and the 150 mg/kg/day group were sectioned, stained with hematoxylin and eosin and examined microscopically. In addition, all main study animals had liver, adrenals, thyroids, ovaries, femurs, spleen and sternum with bone marrow tissue taken and examined microscopically as well.

- liver
- kidneys
- heart
- spleen
- lungs with bronchi
- tongue
- stomach
- duodenum
- jejunum
- ileum
- cecum
- colon
- mesenteric lymph nodes
- submandibular lymph nodes
- thymus
- pancreas
- submandibular glands
- sublingual glands
- Harderian glands
- eyes
- brain
- esophagus
- thyroids and parathyroids
- pituitary
- adrenals
- sciatic nerves
- skeletal muscles (femoral)
- skin
- mammary glands
- femurs (with bone marrow)
- sternum (with bone marrow)
- trachea
- urinary bladder
- ventral prostate
- epididymides
- seminal vesicles
- testes
- vagina
- ovaries
- uterus
- oviducts*
- nose*
- spinal cord (cervical and lumbar)
- aorta
- extraorbital lacrimal glands*
- Zymbal's glands*

Treatment-related histopathologic findings were observed in the liver, adrenal gland, and thyroid of both male and female rats. Female rats demonstrated treatment-related effects in the ovary and spleen, though the latter organ solely by a slight increase in the severity of findings which were observed in all treatment groups including controls. Primary findings in the liver were hepatocellular hypertrophy in the centrilobular regions with dosing ≥ 40 mg/kg/day. An observation of hepatocyte degeneration, scored as mild in severity was observed in a single high dose male and was not observed in other males in this group nor in male controls or in high dose or control females. The adrenal gland of both males and females demonstrated evidence of diffuse cortical vacuolization in males at 150 mg/kg/day and females ≥ 40 mg/kg/day. The thyroid of both males and females demonstrated follicular cell hypertrophy which in males began at 10 mg/kg/day but in females was only observed at 150 mg/kg/day. The incidence and severity (minimal) of follicular cell hypertrophy in males did not increase between 10 and 40 mg/kg/day but was obviously increased at 150 mg/kg/day. In virtually all animals including those in the control groups, microscopic observation of the spleen indicated hematopoietic cell proliferation and pigmentation. This was considered minimal in all male treatment groups but in females became slightly more distinct with some observations scored as mild in severity at 150 mg/kg/day. In females, interstitial cell vacuolization within the ovary was observed at 150 mg/kg/day treatment group only and was scored as minimal to mild in severity. See the table below for incidence.

Microscopic Findings										
	Species					Duration				
	Rat					26 weeks (no recovery groups)				
	Males (n = 15/group)					Females (n = 15/group)				
	Dose Administered (mg/kg/day)					Dose Administered (mg/kg/day)				
ORGAN SYSTEM	C	2.5	10	40	150	C	2.5	10	40	150
Liver										
Hepatocyte degeneration	0	0	0	0	1	0	0	0	0	0

Hepatocellular hypertrophy, centrilobular	0	0	0	5 (min-mild)	14 (min-mild)	0	0	0	4 (min-mild)	15 (min-mild)
Adrenal Gland										
Cortical vacuolization, diffuse	0	0	0	0	8	0	0	0	1	3
Thyroid										
Follicular cell hypertrophy	0	0	1	1	5	0	0	0	0	6
Spleen										
Hematopoietic cell proliferation	13	14	13	15	13	14	14	14	15	14 (min-mild)
Pigmentation	15	15	15	15	15	15	15	15	15	15 (min-mild)
Ovary										
Intracytoplasmic vacuolization	N.A.					0	0	0	0	12 (min-mild)
All findings were considered minimal in severity unless otherwise noted; N.A., not applicable.										

Toxicokinetics:

3 animals/sex/group in the toxicokinetic satellite groups 6-9 had 0.25 mL of blood drawn from the jugular vein at 0.25, 0.5, 1, 3, 6, and 24 hr after the first dose and before dosing and at the same time-points after the 96th and 173rd dose (i.e. during the 13th and 26th week of treatment). TAK-375 was determined in Day 1, 96 and 173 samples while metabolites were assessed only in Day 173 samples. On Days 1 and 96 the method used for quantitation of TAK-375 yielded a LOQ of [] (range []) while for Day 173 the method used for TAK-375 and metabolites had a LOQ of [] (range []) for TAK-375 and [] for the individual metabolites.

As can be seen in the Sponsor's table below, C_{max} and AUC₀₋₂₄ values of TAK-375 and the metabolites M-I, M-II, M-III and M-IV generally increased in a dose-dependent manner. Females demonstrated higher C_{max} and AUC values of TAK-375 and metabolites at virtually all dose levels and time-points (TAK-375) though the M-I metabolite appeared to be higher in males as compared to females at terminal sampling at all dose levels. TAK-375 accumulation was observed to occur in both males and females with dosing ≤ 40 mg/kg/day with lower dose groups showing the greatest degree of accumulation (~4 to 7-fold) decreasing with increase in dose (at 40 mg/kg/day this accumulation was approximately 2-fold in females but 0.6-fold in males though AUC values increased at terminal dose sampling ~2-fold in both males and females). At 150 mg/kg/day, however repeated dosing appeared to produce no accumulation of drug and a reduction in exposure such that AUC values on Day 173 (terminal sampling) was slightly more than half the initial systemic exposure observed after dosing on Day 1. This further supports an induction of hepatic enzymes as an explanation for increased liver weight and histopathologic findings.

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Table 1 Toxicokinetic parameters for TAK-375, M-I, M-II, M-III and M-IV in rats

Dose (mg/kg/day)	Analyte	No. of dosing	Male (n=3)			Female (n=3)			Total (n=6)		
			T _{max} (h)	C _{max} (ng/ml.)	AUC _{0-72h} (ng h/ml.)	T _{max} (h)	C _{max} (ng/ml.)	AUC _{0-72h} (ng h/ml.)	T _{max} (h)	C _{max} (ng/ml.)	AUC _{0-72h} (ng h/ml.)
2.5	TAK-375	1M	0.3 (0.1)	27 (73)	27 (38)	0.3 (0)	93 (32)	40 (10)	0.3 (0.1)	60 (45)	33 (26)
		96h	0.3 (0)	130 (26)	110 (40)	0.3 (0)	397 (116)	250 (61)	0.3 (0)	263 (164)	180 (89)
		173rd	0.3 (0)	193 (71)	204 (70)	0.3 (0)	332 (244)	224 (105)	0.3 (0)	272 (182)	214 (81)
	M-I	173rd	0.3 (0.1)	271 (86)	245 (57)	0.3 (0.1)	481 (145)	276 (41)	0.3 (0.1)	340 (127)	261 (45)
	M-II	173rd	0.3 (0.1)	107 (96)	123 (91)	0.3 (0.1)	327 (104)	146 (125)	0.3 (0.1)	217 (150)	236 (155)
	M-III	173rd	0.3 (0.1)	237 (98)	270 (137)	0.3 (0.1)	473 (151)	353 (131)	0.3 (0.1)	365 (164)	461 (156)
10	TAK-375	1M	0.3 (0)	473 (237)	360 (121)	0.3 (0)	1810 (497)	720 (178)	0.3 (0)	742 (460)	540 (240)
		96h	0.3 (0)	2790 (521)	2007 (493)	0.3 (0)	1740 (382)	1977 (81)	0.3 (0)	2265 (706)	1992 (445)
		173rd	0.3 (0)	1397 (355)	1651 (397)	0.3 (0)	2888 (867)	2236 (476)	0.3 (0)	2242 (909)	1943 (506)
	M-I	173rd	0.3 (0.1)	1519 (165)	2352 (1118)	0.3 (0.1)	1554 (496)	1604 (428)	0.3 (0.1)	1537 (331)	1978 (839)
	M-II	173rd	0.4 (0.4)	248 (14)	723 (152)	0.5 (0)	1152 (372)	1938 (481)	0.5 (0.2)	700 (543)	1335 (731)
	M-III	173rd	0.3 (0.1)	1133 (88)	2349 (718)	0.3 (0)	1918 (1021)	3478 (1121)	0.4 (0.1)	1327 (778)	2914 (1044)
40	TAK-375	1M	0.3 (0.1)	3957 (1030)	4781 (864)	0.3 (0.1)	6317 (1627)	9623 (2350)	0.3 (0.1)	3137 (1774)	7201 (3088)
		96h	0.5 (0)	4193 (1732)	31890 (3146)	0.3 (0)	7903 (7449)	13197 (3763)	0.4 (0.1)	6048 (1789)	12343 (3761)
		173rd	0.4 (0.4)	2456 (332)	2729 (2093)	0.4 (0.1)	18620 (3692)	19437 (7214)	0.5 (0.3)	7063 (3543)	13388 (2976)
	M-I	173rd	0.6 (0.4)	2033 (590)	8464 (1944)	0.5 (0)	3528 (474)	3241 (836)	0.5 (0.2)	3292 (541)	7004 (2080)
	M-II	173rd	1.0 (0)	324 (137)	3282 (1612)	1.0 (0)	2252 (253)	9923 (809)	1.0 (0)	1838 (1506)	6428 (3840)
	M-III	173rd	0.7 (0.3)	2810 (461)	9079 (1835)	1.0 (0)	8193 (1419)	26819 (7491)	0.8 (0.3)	1102 (3517)	17949 (10872)
150	TAK-375	1M	1.0 (0)	223 (26)	1512 (413)	2.3 (1.2)	1210 (162)	7452 (1656)	1.7 (1.0)	716 (550)	4482 (3428)
		96h	0.4 (0.1)	9087 (1469)	47170 (10181)	0.3 (0.1)	21893 (10194)	91880 (16852)	0.4 (0.1)	15490 (9639)	70173 (28139)
		173rd	0.7 (0.3)	7227 (1209)	26917 (16087)	0.5 (0)	15173 (3234)	43267 (14633)	0.6 (0.2)	11200 (4871)	36112 (15830)
	M-I	173rd	0.5 (0)	7623 (1888)	28731 (18244)	0.7 (0.3)	13078 (4839)	37976 (17769)	0.6 (0.2)	10330 (4432)	43364 (22698)
	M-II	173rd	0.5 (0)	7798 (2206)	22091 (7719)	0.4 (0.1)	3802 (691)	10964 (2997)	0.5 (0.1)	1800 (2632)	14528 (8037)
	M-III	173rd	1.0 (0)	794 (276)	3412 (4411)	0.8 (0.3)	3917 (826)	23098 (1988)	0.9 (0.2)	2355 (1804)	14255 (10153)
M-IV	173rd	0.8 (0.3)	4789 (279)	26159 (8544)	0.8 (0.3)	9940 (2829)	72011 (10330)	0.8 (0.3)	7365 (1346)	42086 (26107)	
	173rd	1.0 (0)	263 (67)	3015 (989)	2.3 (1.2)	1255 (178)	10914 (2746)	1.7 (1.0)	730 (537)	4464 (3115)	

Mean (S.D.).

Other: Activity of hepatic drug metabolizing enzymes

Liver samples were taken from 5 animals/sex/main study group for assessment of aminopyrine-N-demethylase activity and aniline hydroxylase activity.

No differences in enzyme activity were found when comparing test-article treated groups and the control group.

Study Title: 4 week Oral Gavage Tox study of TAK375 in monkeys

Study No: M-11-26

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Conducting laboratory and location: Drug Safety Res Labs, Takeda Chemical Industries, Osaka, Japan.

Date of study initiation: Mar 11th 1998

GLP compliance: Yes, FDA and Japan

QA- Report Yes (x) No ()

species/strain: Cynomolgus monkeys

#/sex/group: 3/sex/dose with additional 4/sex/dose for TK

age/weight: 2yrs 11months to 3yrs 2months/2.22-3.53kg m and 2.29-2.81kg f

dosage groups in administered units: 0, 3, 12, 50, 200mg/kg/d administered in the *morning*.

route, form, volume: oral gavage; 10ml/kg; control monkeys received the vehicle: 0.5% methylcellulose solution.

Drug, lot# and % purity: M375-002; $\text{C}_{12}\text{H}_{15}\text{N}_3$ pure.

Formulation/vehicle: drug in 5% methylcellulose (MC) as a suspension.

Observations and Times:

Clinical signs: 1/d am predose and 3times/day (predose and 1-6hr postdose).

Body weights: 2x predose, before dosing on 1st day of dosing, and 1x per wk thereafter.

Food Intake: daily; calculated as the difference between wt of diet given to each monkey and that remaining.

Food intake for the week was calculated.

Water Intake and Urine output: both parameters taken predose and on d17 of dosing. Urine output was collected over 24hr.

Body Temp. and HR: for both parameters, predose, d0, and d22 of dosing at predose, 2, 6, and 24hr postdose

Ophthalmoscopy: predose and on day of autopsy; both direct and indirect measurements were done.

Urinalysis: predose and on d18 of dosing.

Hematology and Clin Chemistry: from all animals (fasted for 18hrs) predose and d23 of dosing.

Liver Enzyme Activity: liver samples were collected from all monkeys in each gr at necropsy.

Gross morphology and Organ wts: on the day after the last dose. all animals from all drug grs were examined.

Histopath: from all animals.

TK: from all animals in each gr after the 1st and 25th dose. Blood collected from the femoral vein predose, 15&30mn, and 1, 3, 6, and 24hr postdose.

Results:

Four-week Oral Gavage Toxicity Study of TAK-375 in Monkeys (Study No. 2700/SU)

Animal	Cynomolgus monkeys, age: 2 years and 11 months - 3 years and 2 months				
Test article	0.5 w/v % MC	TAK-375			
Dosage level (mg/kg/day)	0	3	12	50	200
Dosage volume (ml/kg)	10	10	10	10	10
No. of animals (M:F)	3:3	3:3	3:3	3:3	3:3
Mortality (M:F)	0:0	0:0	0:0	0:0	0:0
Clinical signs	—	—	—	Vomiting # (M:1, F:1)	Vomiting #
Body weight	—	—	—	—	—
Food consumption	—	—	—	—	—
Water intake	—	—	—	—	—
Urine output	—	—	—	—	—
Body temperature	—	—	Day 0: ↓ (M:1, F:1), Day 22: —	Day 0: ↓ (M:1, F:1), Day 22: —	Day 0: ↓ (M:2, F:3), Day 22: —
Heart rate	—	—	Day 0: ↓ (M:1), Day 22: —	Day 0: ↓ (M:1), Day 22: —	Day 0: ↓ (M:1, F:1), Day 22: —
Ophthalmoscopy	—	—	—	—	—
Urinalysis	—	—	—	—	—
Hematology	—	—	—	—	↑ PLT (M:1)
Blood coagulation test	—	—	—	—	—
Blood chemistry	—	—	—	—	↑ ALT # (M:1, F:1)
Plasma T3, T4 levels	—	—	—	—	—
Activity of hepatic drug-metabolizing enzymes	—	—	↑ ADM (F:1)	↑ ADM (M:1, F:1), ↑ AH (M:1)	↑ ADM (M:1, F:2), ↑ AH (M:1, F:1)
Gross pathology	—	—	—	—	—
Organ weights	—	—	—	—	↑ Liver
Histopathology	—	—	—	—	—
Concentrations of TAK-375 in plasma (M:F)					
Tmax (h)	1st	0.3:0.3	0.8:0.4	0.5:0.4	0.7:1.5
	25th	0.5:1.0	0.4:0.7	0.7:0.8	2.7:1.0
Cmax (µg/mL)	1st	0.13:0.07	1.14:1.24	7.03:7.90	10.37:15.39
	25th	0.05:0.01	0.73:0.44	2.73:2.55	9.28:13.12
AUC (µg·h/mL)	1st	0.10:0.05	1.59:1.44	17.96:16.33	53.19:97.65
	25th	0.14:0.04	1.23:0.87	9.06:10.36	75.29:86.73
Conclusion	Non-toxic dose: 12 mg/kg/day				

—: No treatment-related effects, †: Increase, ↓: Decrease, #: Toxicologically significant changes.

Vomiting occurred 1-6hr postdose in the 50 and 200mg/kg dose grs, 1 or more times per day, and seen 1 or 4 days during dosing at 50mg/kg and in 8-27d in the 200mg/kg gr. Incidence increased with increase in dose. Vomiting was also seen during or immediately after dosing in several monkeys; the sponsor however, considered this not to be drug related but the reviewer believes a drug effect can not be excluded. The sponsor indicated that melatonin itself at a single oral gavage dose of 200 or 1000mg/kg also caused vomiting in monkeys (article in preparation; not yet published). There seemed to be tolerance developing for the induced hypothermia as no change was seen with repeated dosing. Platelet count was increased in 1HDM, a finding considered of no tox significance since no histopath findings were found in bone marrow and no thrombotic or coagulation changes were seen. Also, recent studies showed that melatonin may possess a stimulatory effect on platelet production since it normalized platelet count after an oral dose to patients with thrombocytopenia. Levels of ALT were sig increased in 2HD monkeys (2x predose levels); no histopath findings. The increase in liver wt was considered by the sponsor to be adaptive changes due to increase in liver enzyme activities. The mean wt of the heart was increased in 1HDM; histopath exam showed left ventricular myocardial hypertrophy and aggregation of alveolar macrophages in the lung. Some of the macrophages were positive with Berlin blue stain indicative of heart failure cells. The

sponsor dismissed this as a drug related finding because it was not seen in any other monkey of this or other drug groups and it is unlikely that such lesion can develop during a 4wk dosing (the reviewer disagrees with the latter explanation based on experience with other drugs, i.e heart lesions has develop within that time frame). Following single administration, d1, mean max concentration and exposure increased more than the dose ratio between 3 & 50mg/kg but the increase was < the dose ratio between 50&200mg/kg (except for AUC in f). Following repeated dosing on d25, both parameters increased dose dependently but more than proportional. A clear explanation and conclusion could not be made due to the large individual variation in the data. In general, there was no sex difference except in the low dose of 3mg/kg where values were higher in m than f. the sponsor concluded that the NOEL for this study is 12mg/kg/d baed on vomiting and liver enzyme changes at 50mg/kg/d dose.

Study title: A 39-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys

Key study findings:

- Male and female cynomolgus monkeys (n=4/sex/group) were administered TAK-375 at a dose of 0 (0.5 w/v% methylcellulose), 3, 12, 50 or 200 mg/kg/day for 39 months by oral gavage in order to assess the effects of chronic exposure to TAK-375 in this animal model. In addition to the standard clinical, laboratory and post-mortem evaluation, a toxicokinetic analysis of TAK-375 and metabolites including the epimer ratio of the M-II metabolite in circulation as well as an assessment of hepatic enzyme content and activity was conducted.
- Clinical signs were observed with dose levels of 12 mg/kg/day and greater and consisted of a decrease in activity while at a dose level of ≥ 50 mg/kg/day an increased incidence of emesis was noted with dosing. At a TAK-375 dose level of 200 mg/kg/day significant toxicities including uncoordinated movement and lying on one side was noted with both males and females and in 2 males there was a single incidence of reduced muscle tone, oligopnea, loss of consciousness and convulsions. Body temperature was decreased significantly in males at 200 mg/kg/day at the 2 hr observation on Study Week 39 (SW39) only. This was not observed in females at the 200 mg/kg/day or in males or females at an earlier (SW13) observation.
- Hematologic assessment suggested a reduction in APTT in female monkeys significant at the 200 mg/kg/day dose level (-17%) in SW39 and trending downward in males (-12% in SW39) though this was not statistically significant. Sponsor dismissed these findings as incidental but noted that 2 males and 2 females in the 200 mg/kg/day group, as well as 1 male in the 50 mg/kg/day group, exhibited APTT values that were below the Historical Control Data range. This reviewer believes the data supports the interpretation that the reduction in APTT is real at 200 mg/kg/day. Additionally, although group comparisons were not statistically significant, 2/4 males at 200 mg/kg/day were observed to have a platelet concentration that was higher than the historical control range.
- Clinical chemistry assessment indicated a significant increase in ALT in males of the 200 mg/kg/day dose level becoming more severe over the duration of the study (SW39: +445% vs. controls), with a trend towards elevation of this parameter in females at 200 mg/kg/day that did not achieve statistical significant (SW39: +75% vs. controls).

Additionally, male monkeys administered 200 mg/kg/day also demonstrated a significant increase in triglycerides (145-164% vs. controls) during the dosing period which was not observed in female monkeys.

- Liver weight was significantly increased relative to body weight in both males and females (+51% and +39%, respectively) at the 200 mg/kg/day dose level.
- Histopathologic assessment of tissues obtained during necropsy revealed a diffuse hepatocellular hypertrophy in all males and females at the 200 mg/kg/day dose level. The severity was considered minimal to slight and was not considered by the reviewing pathologist to be consistent with hepatic enzyme induction but more reflective of the increases in ALT and triglycerides. This reviewer notes the clinical chemistry results pertained primarily to male monkeys while this microscopic finding was observed in both males and females.
- Toxicokinetic samples taken during SD1, SW13, SW26 and SW39 indicated no evidence of reduced exposure to TAK-375 or metabolites with repeated dosing though the first sample used to determine metabolite concentration was obtained during SW13. Exposure increased with dose in a dose-proportional or greater-than dose-proportional manner. In agreement with previous metabolic studies, the active metabolite M-II is only a very minor metabolite in monkey, having an exposure approximately 0.4 – 1.0% of the parent drug. Assessment of epimer ratio of the M-II metabolite indicated that the (2S, 8S) epimer is predominant (nearly 15-fold higher than [2S, 8R]) in this species.
- Assessment of hepatic microsomal enzymes obtained during necropsy revealed no effect of TAK-375 treatment on cytochrome P450 content or activity of two cytochrome P450-dependent enzymes, aniline hydroxylase and aminopyrine-N-demethylase. Therefore increases in liver weight and histopathology should not be ascribed to adaptive changes in hepatic enzyme status. This finding is further confirmed by the toxicokinetic data demonstrating no auto-induction with repeated TAK-375 administration.
- The NOAEL identified in this study, and with which this reviewer is in agreement, is 12 mg/kg/day due to the clinical signs of excessive emesis observed at 50 mg/kg/day. The exposure to TAK-375 and TAK-375 metabolites at this NOAEL level of 12 mg/kg/day at the end of the study is:

TAK-375		M-I		M-II		M-III		M-IV	
C _{max} (ng/mL)	AUC ₀₋₂₄ (ng•h/mL)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng•h/mL)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng•h/mL)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng•h/mL)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng•h/mL)
1,006	1,626	1,008	1,196	8	13	822	2,378	11	14

Study no.: M-11-232
 Volume #, and page #: 4.2.3.2 Repeat-Dose Toxicity
 Conducting laboratory and location: []
 Date of study initiation: January 19, 1999
 GLP compliance: Yes
 QA report: yes (X) no ()
 Drug, lot #, and % purity: TAK-375, lot #s: M375-006, M375-007, M375-008 (not used),
 [] purity; methylcellulose [] [] lot 709835.

Methods

Doses: Doses used in this study were vehicle (0.5% methylcellulose) or TAK-375 at 3, 12, 50 or 200 mg/kg/day. All doses were within 10% of nominal concentration, stability and homogeneity confirmed as reported in an analytical chemistry report.

Species/strain: Monkey/Cynomolgus (*Macaca fascicularis*)

Number/sex/group or time point (main study): 4/sex/group (main study)

Route, formulation, volume, and infusion rate: Oral solution; 10 mL/kg/day

Satellite groups used for toxicokinetics or recovery: No recovery groups used

Age: 3-4 years at start of study

Weight: 2.0 – 3.9 kg (males); 2.2 – 3.3 kg (females)

Sampling times: Toxicokinetic sampling on Day 1 as well as Weeks 13, 26 and 39.

Samples were collected pre-dose (except for Day 1), 0.25, 0.5, 1, 3, 6 and 24 hr after drug administration.

Unique study design or methodology (if any): No unique aspects of the study design.

Study group design is described below:

Group No. Identification	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Animal Number	
			Males	Females
1 0.5% methylcellulose	0	10	101-104	151-154
2 TAK-375	3	10	201-204	251-254
3 TAK-375	12	10	301-304	351-354
4 TAK-375	50	10	401-404	451-454
5 TAK-375	200	10	501-504	551-554

TK sampling for TAK-375 and metabolites M-I, M-II, M-III and MIV were collected during Weeks 13, 26 and 39. TK sampling for M-II epimers was conducted on week 13 from 1 male in the 50 mg/kg/day group and 1 male and female from the 200 mg/kg/day dose group. Also, 28/44 animals used on study were colony monkeys.

ResultsMortality:

Mortality was assessed twice daily.

There were no unscheduled deaths recorded during this study.

Clinical signs:

Clinical signs were assessed once before treatment and again at 1 and 6 hr after administration of drug. Detailed clinical examinations were performed once prior to the start of dosing and once weekly after initiation of dosing regimen. Body temperature was recorded 4 times during the pretreatment period for each animal and also on Weeks 13 and 39 of the study. Temperature was taken prior to dosing and approximately 2, 6 and 24 hr after drug administration.

Apparent test article-related clinical observations included **frequent emesis** and foamy material noted in the cages of treated animals in all animals but with a much higher incidence in animals receiving oral administration of TAK-375. The Sponsor noted that when "material

was found in the cage tray, the finding was indicated as foamy material since it could be salivation, vomit or refluxed test article". Other clinical signs which appeared to have a relationship to dose level included **decreased activity** with doses ≥ 12 mg/kg/day, and **salivation** observed with dosing ≥ 50 mg/kg/day. Several clinical observations were only reported for animals at the 200 mg/kg/day dose level and included slight to moderate **uncoordination** in both males and females and **lying on the side** in both males and females. Additionally, two males at the 200 mg/kg/day level were noted to have **decreased muscle tone, abnormal breathing considered shallow and weak with loss of consciousness and convulsions**. These two male monkeys only displayed these findings once during the 9-month treatment portion of the study, once on day 69 in one animal (animal #501) and on day 135 in the other (animal #504).

Body weights:

Body weight was taken three times prior to the onset of dosing and once weekly during the dosing period. Animals were fasted overnight at the end of study with weight recorded prior to sacrifice and necropsy.

Statistically significant increases and decreases in body weight gain were sporadically noted between TAK-375-treated groups and controls during the study. These were considered incidental as they were not clearly dose-related or found consistently throughout the study. Body weight by the end of the study was not affected by treatment with TAK-375 when compared with body weight of vehicle treated animals.

Body Weight (end of study) vs. Controls					
	Dose (mg/kg/day)				
	0	3	12	50	200
Males	-	+6%	-1%	-3%	+1%
Females	-	-5%	+1%	0	+1%

Food consumption:

Food consumption was assessed daily for the week prior to initiation of dosing and daily food consumption was recorded throughout the study. Data was recorded as analyzed as consumption per week, however.

There was no treatment-related effect of TAK-375 on food consumption.

Body Temperature:

Body temperature was recorded during the pretreatment period and during weeks 13 and 39 at 2, 6 and 24 hr after dosing.

A statistically significant decrease in body temperature was observed for male monkeys at the 200 mg/kg/day dose level when compared with control in SW39 (final week) at the 2 hr post-dose observation only (38.6°C vs. 39.3°C). No differences in body temperature were noted during Week 13 in males or females at any dose level compared with control. The absence of this finding in females at 39 weeks and in both sexes at week 13 suggests this finding is not toxicologically relevant though it is conceivable this could be related to the pharmacologic effect as a melatonin receptor agonist as melatonin itself is capable of reducing body temperature.

Ophthalmoscopy:

An ophthalmologic examination was conducted once prior to the initiation of dosing and three times during the study (Weeks 13, 26 and 39). Examination was conducted using direct, indirect and slit-lamp methods by a board-certified veterinary ophthalmologist.

There were no treatment-related effects on ophthalmologic exam. Minor findings were observed in all treatment groups including controls and are commonly found in this species.

EKG:

An ECG recording was performed on all animals using 6 limb leads once prior to the initiation of dosing and during the study on Days 1 (2 hr after first dose), and Weeks 4, 13 and 39. The recording was reviewed by a board-certified veterinary cardiologist consultant. At the same time, blood pressure was taken utilizing a brachial cuff.

One monkey receiving the 3 mg/kg/day dose exhibited a single ventricular ectopic beat during "Week 12" which this reviewer interprets as being SW13. All other records from this monkey and the remaining animals in all groups were considered to be within normal limits. The external cardiologist's report concludes (p. 40):

There was no electrocardiographic evidence of test article action in this study. A single ventricular ectopic beat in one monkey in a study of this size has no significance for the study since this is a common spontaneous arrhythmia in monkeys.

Review of blood pressure readings from SD1, SW4, SW13 and SW39 indicates a significant reduction in blood pressure in the 200 mg/kg/day group females on SW4 at 2 hr post drug administration. No effect was observed in males at the 200 mg/kg/day dose level and this finding was not observed in 200 mg/kg/day females when observations were taken on the other three occasions during the study. Blood pressure was highly variable and this reviewer considers this to be an incidental finding.

Clinical Pathology:

Clinical pathology parameters were obtained once prior to initiation of dosing and during the study in Weeks 13, 26 and 39. Animals were fasted overnight prior to blood sampling to stabilize clinical biochemistry values.

Hematology:

Blood was obtained as described above with the following parameters examined:

erythrocyte count	mean corpuscular hemoglobin (MCH)
leukocyte count	mean corpuscular hemoglobin concentration (MCHC)
platelet count	mean platelet volume
hematocrit value	reticulocyte count
hemoglobin concentration	prothrombin time
mean corpuscular volume (MCV)	activated partial thromboplastin time

Males exhibited a significant increase in PT at a dose of 3 and 50 mg/kg/day on SW13 observation but as this observation was not dose-dependent or durable it is considered to be incidental by both Sponsor and this reviewer. A mild reduction in activated partial thromboplastin time (APTT) which did not achieve statistical significance was noted by this reviewer in all TAK-375-treated males. APTT was reduced -12% in the 200 mg/kg/day males at SW39 when compared with the values from controls. In particular, the Sponsor notes that 1 male at 50 mg/kg/day and 2 males at a dose of 200 mg/kg/day had APTT values that fell below the Historical Control Data for this species in their laboratory.

Females exhibited a significant increase in reticulocytes in the 50 mg/kg/day group at SW26 but this was not observed at higher dose levels or at later observation and was considered incidental. A decrease in reticulocyte count noted in the 3, 12 and 200 mg/kg/day group females during SW39 appears to be an artifact caused by 2 unusually high counts in control animals. Prothrombin time (PT) was, however, decreased at a dose of 200 mg/kg/day during SW39. APTT, likewise, was significantly decreased at multiple observations in the 3 mg/kg/day (SW13 and SW39) and 200 mg/kg/day group (all observations). Although the Sponsor dismissed these reductions as incidental, they noted that 2 females in the 200 mg/kg/day group were outside the historical range for this parameter. When the values were evaluated as a change vs. controls by the Sponsor and change vs. pre-dose values by this reviewer, the following table was generated.

Hematology					
APTT [Females]					
Sec (% change from control)					
Dose	0	3	12	50	200
Week					
Pre-Dose	21.3 sec	19.6 (-8%)	23.1 (+8%)	22.6 (+6%)	20.7 (-3%)
SW13	22.3	19.3 (-13%)*	20.9 (-6%)	21.3 (-4%)	18.4 (-17%)**
SW26	20.8	19.0 (-9%)	19.5 (-6%)	19.8 (-5%)	18.0 (-13%)*
SW39	19.7	17.7 (-10%)*	19.6 (-1%)	18.7 (-5%)	16.4 (-17%)**
* p<0.05, ** p<0.01					
APTT [Females]					
Sec (% change from pre-dose)					
Dose	0	3	12	50	200
Week					
Pre-Dose	21.3 sec	19.6	22.6	23.1	20.7
SW13	22.3 (+5%)	19.3 (-2%)	20.9 (-10%)	21.3 (-6%)	18.4 (-11%)
SW26	20.8 (-2%)	19.0 (-3%)	19.5 (-16%)	19.8 (-12%)	18.0 (-13%)
SW39	19.7 (-8%)	17.7 (-10%)	19.6 (-15%)	18.7 (-17%)	16.4 (-21%)

The decrease in APTT values for the 3 mg/kg/day and 200 mg/kg/day group detected when compared with control values (upper table) appeared throughout the dosing period of the study while the 12 and 50 mg/kg/day values were not significantly different. It should be noted, however, that these mid-dose groups began with a higher APTT time than controls which may explain the lack of statistical significance in these groups. When APTT values were assessed as a %change from pre-dose values (lower table) it can be observed that 1) there is a general decline in APTT values over the dosing period in all groups, including control females, but 2) a dose-dependent effect of TAK-375 treatment would appear to occur such that APTT values decline more greatly in groups given higher TAK-375 doses. Though the Sponsor dismisses the APTT data as incidental and the group means are within the Historical Control Data range the Sponsor did also note that 1 male in the 50 mg/kg/day group and 2 males and 2 females in the 200 mg/kg/day group exhibited an APTT value that was below the HCD range. Taken together this suggests a small but real effect of TAK-375 or its metabolites on this parameter.

Clinical chemistry:

Blood was obtained as described above with the following clinical chemistry parameters analyzed:

A/G ratio (calculated)	calcium	glucose	total cholesterol
alanine aminotransferase (ALT)	chloride	inorganic phosphorus	triglyceride
albumin	creatinine	potassium	urea nitrogen
alkaline phosphatase (ALP)	creatine kinase (CK)	sodium	lactate dehydrogenase (LDH)
aspartate aminotransferase (AST)	globulin	total bilirubin	total protein

Males demonstrated significantly increased ALT in the 200 mg/kg/day dose level at all treatment observations (SW13, SW26 and SW39) over 4-fold and was elevated over pre-dose values (also up to 4-fold) in this group as well. TAK-375-treated groups exhibited higher pre-dose ALT values (not significant, however) when compared to control animals but the elevation of ALT in the 200 mg/kg/day group males was durable and appeared to increase in severity over time as shown in the table below.

Hematology					
Week	Dose	ALT (males)			
		U/L (%change vs. control)			
	0	3	12	50	200
Pre-Dose	28.3	61.8 (+118%)	40.8 (+44%)	59.3 (+110%)	43.3 (+53%)
SW13	36.0	57.8 (+61%)	49.0 (+36%)	61.8 (+72%)	104.5 (+190%)**
SW26	37.5	51.5 (+37%)	36.5 (-3%)	57.5 (+53%)	108.8 (+190%)**
SW39	43.8	54.0 (+23%)	40.5 (-8%)	47.0 (+7%)	238.8 (+445%)**

** p<0.01

Also in males, a significant increase in triglycerides was observed in the 200 mg/kg/day dose group (SW13, SW19 with SW26 elevated but not significant). The increase in triglycerides did not worsen over the duration of the study, however, and was maximally increased 145-164% vs. controls (51-71% above pre-dose values).

Data from females was suggestive of a mild increase in ALT in the 200 mg/kg/day group in 3/4 monkeys (+75% vs. control values at SW39) though this was not statistically significant. Sporadic statistically significant findings of reduced total bilirubin was noted in the 50 and 200 mg/kg/day groups but only on SW13 (-36% and -40%, respectively) though values were generally lower in the TAK-375-treated groups when compared with both controls and pre-dose values. Total bilirubin values did not worsen in the TAK-375-treated female monkeys over the duration of the study, however.

Occasionally, values for lactate dehydrogenase, creatinine kinase, blood urea nitrogen, total protein, A/G ratio, globulin, potassium, and chloride attained statistical significance but these were sporadic, not dose-dependent or durable, and/or found in one gender and are considered to be incidental findings by both Sponsor and this reviewer.

Urinalysis:

Urine was obtained at the time-points described above and the following urine parameters were analyzed:

bilirubin	nitrite
blood	pH
color and appearance	protein
glucose	specific gravity
ketones	urobilinogen
microscopy of centrifuged deposit	volume

No abnormal findings were observed in either male or female monkeys.

Gross pathology:

Animals were fasted overnight and euthanized using an i.v. administration of sodium pentobarbital followed by exsanguination of the axillary or femoral arteries. Ketamine-HCl for injection, USP was given intramuscularly prior to moving the animals from cages to the necropsy area. Gross pathology was conducted immediately on all animals after sacrifice. Necropsy was conducted under the supervision of a pathologist and included an external examination and detailed internal examination.

No treatment-related macroscopic effects were noted during the necropsy examination.

Organ weights:

The organs listed below were collected for analysis of weight. Paired organs were weighed together and organ weights to terminal body weights were calculated as well.

adrenal glands	pituitary
brain	prostate
epididymides	spleen
heart	submandibular gland
kidneys	testes
liver	thymus
lungs	thyroid lobes and parathyroid glands
ovaries	uterus
pancreas	

Absolute organ weights were not different in any TAK-375-treated group when compared with control animal organ weights.

The liver was significantly increased in weight when adjusted for body weight in both males and females at the 200 mg/kg/day dose level, however.

Organ Weight					
Liver weight (relative)					
% change vs. control					
Dose (mg/kg/day)	0	3	12	50	200
Males	-	-10%	+3%	+17%	+51%**
Females	-	0	-1%	+3%	+39%**

** p<0.01

Histopathology: Adequate Battery: yes (X), no ()
Peer review: yes (X), by Sponsor

Abnormalities	Kidneys	Skeletal muscle (thigh)
Animal identification	Lacrimal glands	Skin (ventral thoracic)
Adrenals	Liver (2 lobe samples) ^b	Spinal cord (cervical and lumbar)
Aorta (thoracic)	Lungs (all lobes) ⁺⁺	Spleen
Brain (and subsections)	Lymph nodes (submandibular and mesenteric)	Sternum (w/bone marrow)
Cecum	Mammary gland ⁺	Stomach
Colon	Optic nerves ^{**}	Sublingual glands
Duodenum	Ovaries	Submandibular glands
Epididymides*	Oviduct ^a	Testes*
Esophagus	Pancreas	Thymus
Eyes*	Pineal gland	Thyroid lobes (and parathyroids) ⁺
Femurs (w/bone marrow)**	Pituitary	Tongue
Gallbladder	Prostate	Trachea
Heart	Rectum	Urinary bladder
Ileum	Sciatic nerve	Uterus
Jejunum	Seminal vesicles	Vagina

*, fixed in Zenker's fluid; **, bone decalcified prior to sectioning; ⁺ examined histopathologically only if present in routine sections, ^{**}, infused with neutral buffered 10% formalin; ^a, retained but not processed; ^b, samples retained for hepatic drug metabolizing enzyme assay

All tissues were fixed in 10% neutral buffered formalin unless stated otherwise. For all animals, 3 sternal bone marrow smears were prepared, one of which was stained with May-Grunwald-Giemsa. These were retained but not evaluated.

All tissues were prepared for examination by embedding in paraffin wax, sectioned and stained with hematoxylin and eosin. A peer review was performed by the Sponsor.

The following table details microscopic findings which were observed to occur at a higher incidence in any TAK-375-treated group than the respective controls irrespective of the presence of dose-dependence.

Histopathology										
	Incidence of Microscopic Findings									
	Dose (mg/kg/day)									
	Males					Females				
	0	3	12	50	200	0	3	12	50	200
# examined[†]	4	4	4	4	4	4	4	4	4	4
Organ/Finding										
Adrenal										
Hyperplasia, cortex	0	0	1	0	0	0	0	0	0	0
Mineralization	0	0	0	0	0	0	0	0	0	1
Liver										
Hypertrophy: hepatocellular (Minimal-Slight)	0	0	0	0	4	0	0	0	0	4
Lipidosis, subcapsular	1	0	0	0	0	0	0	0	0	1
Lung										
Pleural fibrosis	0	1	2	0	2	0	1	1	0	1
Alveolar histiocytosis	0	1	1	1	2	1	0	0	1	1
Brain										
Prominent glial cells	0	0	0	0	0	0	0	0	1	1

Heart										
Inflammation	0	0	0	0	0	0	0	0	1	0
Sciatic nerve										
Hemorrhage	0	0	1	0	0	0	0	0	0	0
Ovary										
Cyst	N/A	N/A	N/A	N/A	N/A	0	0	0	2	1
Pineal gland										
Pigment deposits	0/3	0/3	1/4	2/4	1/4	0/4	1/4	2/4	1/4	2/4
Prostate										
Immature	1	1	3	2	1	NA	N/A	N/A	N/A	N/A
Rectum										
Degeneration of muscle	0	0	0	0	1	1	0	0	0	1
Erosion	0	0	0	1	0	0	0	0	0	1
Seminal vesicle										
Immature	1	1	0	2	1	N/A	N/A	N/A	N/A	N/A
Thymus										
Atrophy	0	1	0	0	2	0	0	0	1	0
Extravasation of Blood	0	1	1	0	0	0	0	0	0	0
Cyst	0	0	0	0	0	0	0	0	0	1
Thyroid										
Thymic ectopia	0	0	0	0	0	0	0	0	0	2
Urinary bladder										
Transitional cell hyperplasia	0	0	0	0	1	0	0	0	0	0
†, All findings graded as minimal in severity unless otherwise specified										
†, number of tissues examined is 4/group unless otherwise specified										

The only clear finding presenting in both male and female monkeys and with an apparent relationship to dose is a **diffuse hepatocellular hypertrophy of the liver**, minimal or slight in severity and observed only at a dose of 200 mg/kg/day. According to the Study Pathologist (p. 50):

Hepatocellular hypertrophy was not clearly zonally distributed and was accompanied by an increased prominence of pale cytoplasm with a degree of fine vesiculation. This change was predominantly centrilobular or centrilobular and midzonal in distribution. Hypertrophy was not seen in any animals from lower dosage groups, nor in control group animals

This finding is in accordance with the observation of increased (relative) liver weight though no evidence was demonstrated to indicate hepatic enzyme induction as the source of this finding.

Observations of pigment deposits were found in the brain (infundibular process), pineal gland, pituitary, lung, stomach, cecum and mesenteric lymph node of one or both sexes but did not show a clear relationship to dose. Other findings noted with a higher incidence in TAK-375-treated groups when compared to vehicle control groups were observed only one gender and/or did not show evidence of a relationship to TAK-375 dose level and therefore are considered likely to be incidental findings.

Toxicokinetics:

Approximately 0.8 mL of blood per time-point per animal was taken from the femoral vein of all treatment animals on SD1, and SW13, SW26 and SW39 (n=4/timepoint/group).

Timepoints assessed were: prior to the dose administration (with the exception of SD1), and

after dosing at 0.25, 0.5, 1, 3, 6, and 24 hr. Concentrations of TAK-375 were determined by [] for SD1 while during SW13, SW26, and SW39 TAK-375 and metabolites M-I, M-II, M-III and M-IV were determined using [] method. Blood samples taken from the control animals which did not receive test-article were disposed without analysis. Additionally, during SW13, the plasma from 3 animals (1 50 mg/kg/day male at 1 hr post-dose and 1 50 mg/kg/day male and female at 1 and 3 hr post-dose) were analyzed for epimers of the M-II metabolite.

TOXICOKINETIC SUMMARY											
Dose	Study Timepoint	TAK-375		M-I		M-II		M-III		M-IV	
		C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·h/mL)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·h/mL)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·h/mL)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·h/mL)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·h/mL)
3	SD1/SW13 [†]	33	23	225	36	0	0	29	56	0	0
	SW39	56	78	241	258	0	0	63	153	0	0
12	SD1/SW13 [†]	704	1,134	1,395	3,523	7	19	691	1,879	23	47
	SW39	1,006	1,626	1,008	1,196	8	13	822	2,378	11	14
50	SD1/SW13 [†]	4,632	12,481	2,475	6,402	27	72	1,792	8,657	96	524
	SW39	4,227	9,923	2,323	7,218	37	102	4,630	24,008	84	448
200	SD1/SW13 [†]	11,272	71,441	3,062	22,655	36	273	5,596	48,829	124	1,052
	SW39	15,311	92,417	3,188	14,841	64	435	4,772	52,630	119	1,394

[†], the first TK observation for TAK-375 was on Study Day 1 (SD1) while for metabolites it was during Study Week 13 (SW13)

Data indicate little reduction in exposure to TAK-375 or the metabolites with repeated dosing over 39 weeks. Evidence of hepatic enzyme auto-induction is absent though the first assessment of TAK-375 metabolite levels occurred on SW13 leaving open the possibility that auto-induction could have occurred prior to this point and is not captured by the analysis time-points. Accumulation of drug with repeated dosing was not observed nor was an effect of gender on exposure to drug or metabolite. Exposure to TAK-375 and metabolites increases with increasing dose in a dose-proportional (M-I) or greater than dose-proportional (TAK-375, M-II, M-III, and M-IV) manner in the monkey.

Assessment of the epimer ratio of circulating M-II metabolite after TAK-375 administration in SW13 revealed the (2S, 8S) epimer (T-163302) is the predominant epimer generated as shown in the following table reproduced from the Sponsor's submission (p. 152)

Table Epimer ratios of T-163302 to T-163306 in monkey plasma - Week 13

Dose (mg/kg/day)	Sex	Animal No.	Time after dosing (h)	Epimer Ratio (%)	
				T-163302	T-163306
50	Male	482	1	82.8	17.2
	Male	501	1	82.7	17.3
200	Female	501	1	95.8	4.2
		554	1	80.5	19.5
		554	1	94.7	5.3

Other:

Activity of Hepatic Drug-Metabolizing Enzymes:

The activity of aminopyrine-N-demethylase and aniline hydroxylase activities were measured from liver samples taken during necropsy and were used to judge general activation of hepatic metabolism.

Assessment of hepatic enzymes using liver microsomes obtained from monkeys after 39 weeks of treatment with TAK-375 at 3, 12, 50 or 200 mg/kg/day revealed no increase in cytochrome P450 content and no increase in activity in two cytochrome P450-dependent

enzymes - aniline hydroxylase and aminopyrine-N-demethylase - when compared to microsomes obtained from vehicle-treated monkeys.

3. Preliminary 1wk i.v. toxicity study of M-II(2S,8S)(T-163302)*, a metabolite of TAK375 in monkeys/M-11-156/study# 3084/SU

Conducting lab: Takeda, Japan

GLP: NO

QA report: NO

Initiation/Termination dates: Jun 1999/Aug 1999

Drug lot#/purity: B13183-48-06

Strain/# per sex per dose: cynomolgus monkeys 2/sex/dose.

Doses & vehicle: 1, 3, 10mg/kg/d and the control group received the vehicle: 30% PEG400.

Administration volume: 4ml/kg/d at i.v. rate of 2ml/min.

Route/Duration: daily i.v. for 1wk

B.wt/age at initiation: 3yr 2month m and 4yr 3month f/2.72-4.39kg males and 2.76-3.26kg females.

* M-II is 2 diastereomers, the M-II(2S,8S)(T-163302) and M-II (2R,8S)(T-163306). — % of total plasma M-II in humans is the M-II(2S,8S). The sponsor conducted this study because the safety of M-II was not adequately assessed when plasma drug levels were determined during wk13 in a 39wk oral gavage tox study of the parent in monkeys. In that study, M-II levels were low compared to the parent TAK-375 levels which were much higher. Therefore, M-II (2S,8S) was given i.v. in this study.

Parameters assessed:

Mortality & Clinical Signs: 3x daily (predose and at 1 & 6hr postdose).

B.wt: 1st day of dosing and 6.

Food intake: daily.

Hematology & Blood Chemistry: day 7: standard assessment for both parameters.

Organ wts: absolute and relative wts of the following organs were determined at necropsy: brain, thymus, heart, lungs, liver, spleen, kidneys, testes/ovaries, pituitary, adrenals, and prostate.

Gross necropsy: standard exam.

Histopath: adrenals, liver, and ovaries.

TK: for T-163302 and T-163306 at 0.25, 1, 2, 4, 8, and 24hr postdose after 1st dose.

⌊

⌋ was the analytical method used. ql= ⌊ ⌋

Results:

There were no compound related findings on any of the parameters measured except for some reddening at injection site observed in all groups including control. Mean C_{0h} and $C_{0.25h}$ and AUC_{0-24hr} increased proportional to dose in females between low and mid dose and slightly more than proportional between mid and high dose (at the 0.25hr concentration and 0-24hr exposure)(table below from sponsor). In males however, the increase in these parameters was less than proportional between low and mid dose and > proportional between mid and high doses.

In general, there was no sex difference in TK. Also noted is higher concentrations of M-II at 0.25hr postdose than values immediately at end of administration. The sponsor indicated, that M-II $C_{0.25hr}$ and exposure at the 10mg/kg in this study represented 59 and 25 folds higher than C_{max} and exposure following 16mg/d clinical dose respectively, (sponsor's statement, no clinical data were provided) .

Toxicokinetic parameters for T-163302 in monkeys - after the 1st dose

Dose (mg/kg/day)	Male (N=2)			Female (N=2)			Total (N=4)		
	C_{0h} (ng/mL)	$C_{0.25h}$ (ng/mL)	AUC_{0-24h} (ng·h/mL)	C_{0h} (ng/mL)	$C_{0.25h}$ (ng/mL)	AUC_{0-24h} (ng·h/mL)	C_{0h} (ng/mL)	$C_{0.25h}$ (ng/mL)	AUC_{0-24h} (ng·h/mL)
1	469	679	729	353	563	623	412 (313)	595 (316)	576 (327)
3	816	1036	1333	904	1332	1924	893 (389)	1287 (332)	1624 (383)
10	4332	5914	13282	2843	7219	8375	2593 (971)	4186 (3576)	10931 (4100)

Mean values: Male, Female, Total: Mean (S.D.) * Immediately after the end of the injection. # Time after the end of the injection.

Summary and Conclusion:

Daily i.v. injection of M-II (2S,8S), a TAK-375 metabolite, to male and female monkeys did not cause deaths or any adverse compound-related findings up to 10mg/kg/d. Mean plasma concentration at 0 and 0.25hr post dosing as well as AUC_{0-24hr} increased non-linearly with dose mainly in males (somewhat linear in females), and was not different between sexes. The sponsor stated that the $C_{0.25hr}$ and exposure at 10mg/kg represent 59 & 25 times higher than these parameters after 16mg/d clinical dose, respectively. The NOAEL for M-II in this study is >10mg/kg/d via i.v. injection.

4. 4wk i.v. toxicity study of M-II(2S,8S)(T-163302), a metabolite of TAK375 in monkeys/M-11-156/study# SBL28-92/M-11-00192

Conducting lab: Takeda, Japan

GLP: Yes/Japan

QA report: Yes

Initiation/Termination dates: Aug 1999/Jan 2000

Drug lot#/purity: M375-M201

Strain/# per sex per dose: cynomolgus monkeys 3/sex/dose.

Doses & vehicle: 1, 3, 10mg/kg d; the control group received the vehicle: 30% PEG400.

Administration volume: 4ml/kg/d at i.v. rate of 2ml/min injected into the cephalic vein of the forearm or the saphenous vein.

Route/Duration: daily i.v. for 4wks

B.wt/age at initiation: male: 4-6yr. female: 3-4yr/3.48-4.33kg males and 2.54-3.68kg females.

Parameters assessed:

Mortality & Clinical Signs: 3x daily (predose, 1 & 6hr postdose).

B.wt: 1st day of dosing and days 6, 13, 20, and 27.

Food intake: daily and reported weekly.

Water Intake: intake over 24hr per monkey, was calculated from the wt (g) of the water provided and that remaining in plastic bottles at predose and end of dosing.

Body Temperature: rectal temp of each conscious monkey was measured using electronic thermometer once predose and at 2, 6, 24hr postdose on days 1&26.

EKG & HR: the former was recorded for each animal once predose and just before dosing and at 2hr postdose on d26; Leads I, II, III, aVr, aVL, and aVf. PR, QT, QRS and QTC were examined using Lead II (QTc was calculated as follows: $QTc = QT/\sqrt{RR}(\text{sec})$).

Ophthalmology: both gross (pen light) and slit-lamp exam predose and wk3 of dosing. Ocular fundi of all monkeys under anesthesia, were examined using a fundus camera predose and wk3 of dosing.

Urinalysis: predose and wk4 at 2hr predosing. Standard parameters determined. Urine volume over 16hr was also measured.

Hematology & Blood Chemistry: pre treatment and wk4 (d27) prior to dosing from femoral vein of each 18-22hr-fasted monkey; standard parameters were determined.

Organ wts: absolute and relative wts of the following organs were determined at necropsy: brain, thyroids, thymus, heart, liver, spleen, kidneys, testes/ovaries, seminal vesicles, epididymides, uterus, pituitary, adrenals, and lungs.

Gross necropsy: standard exam.

Histopath: all tissues/organs from all monkeys were stained and examined under LM.

Liver Enzymes: at necropsy portion of the right lobe from each monkey was excised and processed for liver enzyme activity: aminopyrine-N-demethylase and aniline-p-hydroxylase.

TK: concentrations of T-163302 and its epimer, T-163306, were measured in plasma from all monkeys. Blood collected from femoral vein immediately after dosing and at 0.25, 0.50, 1, 2, 4, 8, and 24hr postdose on days 0 & 27. Σ J was the analytical method used. $ql = \Sigma$ J

Results:

Similar to the preliminary tox/TK i.v. study above, there were no compound related findings in any group up to 10mg/kg/d dose. Any findings that reached statistical significance were within normal range for cynos or were not dose dependent. Mean C_{0tr} and AUC_{0-24hr} increased proportional to dose in both sexes and were similar after either single or repeat dosing (table below from sponsor). There seem to be no sex differences (though large animal variations were seen). The sponsor indicated that the C_{0tr} and AUC_{0-24hr} represent 60 and 20 times the expected M-II (2S.8S) exposures when the clinical dose of TAK375 orally is 16mg/d. The other isomer, M-II (2R.8S), was below the ql of Σ J and was not detectable at any time point.

Toxicokinetic parameters for T-163302 in monkeys

Dose (mg/kg/day)	No. of dosing	Male (N=3)		Female (N=3)		Total (N=6)	
		C _{0h} (ng/mL)	AUC _{0-24h} [§] (ng·h/mL)	C _{0h} (ng/mL)	AUC _{0-24h} [§] (ng·h/mL)	C _{0h} (ng/mL)	AUC _{0-24h} [§] (ng·h/mL)
1	1st	1107 (299)	913 (15)	1031 (211)	562 (27)	1069 (235)	737 (193)
	28th	919 (125)	855 (208)	1047 (354)	657 (137)	983 (248)	756 (191)
3	1st	2974 (640)	2145 (178)	2834 (392)	1625 (367)	2904 (481)	1885 (384)
	28th	2691 (520)	1917 (175)	2574 (854)	1619 (627)	2633 (635)	1768 (443)
10	1st	8487 (667)	10010 (4945)	8431 (1372)	6697 (1231)	8459 (966)	8353 (3699)
	28th	8785 (735)	9783 (2664)	7833 (1563)	7610 (690)	8309 (1216)	8696 (2108)

Mean values: Male, Female, Total: Mean (S.D.). *: Time after the end of the injection. §: AUC from 0 to 24 h after the end of the injection.

Summary and Conclusion:

Daily i.v. injection of M-II (2S,8S) to male and female cynomolgus monkeys did not cause death or other adverse effects up to 10mg/kg/d and 4wks of injection. Mean concentration and exposure increased linearly with dose however, this was not the case in the above 1wk i.v. study at the same doses with the exception of females. Also, there was no drug accumulation with dosing and no sex difference (although there were large inter animal variations to accurately make the latter conclusion). The C_{0hr} and AUC_{0-24hr} mean values at the 10mg/kg were 8459ng/ml and 8309ng.hr/ml (combined sexes, values for days1&27) and 8353ng/ml and 8696ng.hr/ml (combined sexes, values for days1&27) respectively. These values represent approximately, 60 & 20 times those *expected* in humans following oral dose of TAK-375 at 16mg/d. The NOAEL in this study is >10mg/kg (similar to the conclusion for the 1month study above).

Study title: Four-Week Oral Gavage Range-Finding Toxicity Study of TAK-375 in Mice

Key study findings:

- The purpose of this 4-week oral toxicity study in the mouse was to serve as a dose range-finding study for a subsequent 13-week range-finding toxicity study from which the dosing of the 2-year carcinogenicity bioassay in the mouse would be determined.
- Mortality and severe clinical signs observed in males at 1500 mg/kg/day in the main study and in both males and females in the satellite toxicokinetic study. Clinical signs included lying on one side, reduced activity, prone position and bradypnea at 1500 mg/kg/day and prone position also observed at 500 mg/kg/day.
- Cholesterol and ALT were increased in both males and females at 1500 mg/kg/day though these increases were less than 2-fold.
- Increases in liver weight, size, along with hepatocellular hypertrophy and evidence of significant reduction in TAK-375 exposure with repeated administration of drug suggest an induction of hepatic metabolizing enzymes and are not considered toxicologically significant.
- Toxicokinetic analysis demonstrated dose-related increase in exposure with increasing dose with a significant reduction in exposure with repeated drug administration which appeared greater in the female. Therefore, exposure appeared slightly higher in the male at all dose levels (max 80% higher than females) with repeated dosing.

- The NOAEL for the study is identified at 150 mg/kg/day ($C_{max} = 5.72 \mu\text{g/mL}$; $AUC_{0-24} = 7.0 \mu\text{g}\cdot\text{h/mL}$) and the MTD appears to be 500 mg/kg/day ($C_{max} = 4.43 \mu\text{g/mL}$; $AUC_{0-24} = 30.3 \mu\text{g}\cdot\text{h/mL}$).

Study no.: M-11-00609

Module: 4.2.3.4.1 Long Term Studies

Conducting laboratory and location: Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd. Osaka, 532-8686, Japan

Date of study initiation: September 7, 1998

GLP compliance: No

QA report: No

Drug, lot #, and % purity: TAK-375, Lot# M375-005, purity < 1 with COA located in Appendix. Content of the test article in the dosing suspension (0.5 w/v% MC) acceptable at initiation and conclusion of study as described in report located in Appendix.

Methods

Doses: 0 (0.5 w/v% methylcellulose), or TAK-375 50, 150, 500 or 1500 mg/kg/day

Species/strain: B6C3F₁ mice

Number/sex/group or time point (main study): 5 mice/sex/group

Route, formulation, volume, and infusion rate: Oral gavage of test article suspension, 10 mL/kg.

Satellite groups used for toxicokinetics or recovery: 29 mice/sex/group for TK

Age: 6 weeks at study start

Weight: 21-24g, males; 18-20g, females

Sampling times: Plasma concentration for TAK-375 parent obtained after the 1st and 29th dose before dosing, and at 0.5, 1, 6, and 24 hr after drug administration.

Unique study design or methodology (if any): Statistical analysis was not conducted on data obtained in this study.

Observation Times & Results

Mortality: Examined daily. Three males in the main study 1500 mg/kg/day group were found dead (2M on Day 1, 1M on Day 2) while 9 satellite males at this dose were also found dead (5M on Day 0, 4M on Day 1). Females in the main study did not demonstrate mortality but 6 satellite females at the 1500 mg/kg/day group were found dead (4F on Day 0, 2F on Day 1) during the study.

Clinical signs: Twice daily or more. Decreased locomotor activity was observed in both males and females with the 1500 mg/kg/day dose. Additionally, prone position in males ≥ 500 mg/kg/day and females at 1500 mg/kg/day and bradypnea in both males and females at 1500 mg/kg was observed. No clinical signs were observed in the 150 mg/kg/day group for either sex.

Body weights: Obtained on Day 0, 3, 7, 10, 14, 17, 21, 24 and 28 of study. No differences in body weight were observed when comparing TAK-375 treatment groups versus control animals.

Food consumption: Once weekly. No differences in food consumption were observed when comparing TAK-375 treatment groups versus control animals.

Ophthalmoscopy: Not done.

EKG: Not done.

Hematology: RBC, WBC, Platelet, hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, reticulocytes. Platelets were observed to be increased in males in the 500 mg/kg/day group and both sexes at 1500 mg/kg/day (↑21% – 38%). Additionally, RBC and hemoglobin levels in the female were elevated by 7-8% and leukocyte concentration was reduced by 33% in the 1500 mg/kg/day group females when compared with control values.

Clinical chemistry: Total protein, albumin, A/G ratio, glucose, total cholesterol, urea nitrogen, Creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase. A/G ratio was decreased dose-dependently in all males groups (max ↓18%) and in females ≥ 500 mg/kg/day (↓19% max). Interestingly, albumin was decreased in both males and females at 500 mg/kg/day but was not as markedly decreased in the 1500 mg/kg/day group. Total cholesterol and ALT were increased in both males and females at 1500 mg/kg/day but were not different from controls in lower dose groups.

Urinalysis: Not done.

Gross pathology: Enlargement of the liver in two males and all females in the 1500 mg/kg dose level.

Organ weights: Liver only. A dose-dependent increase in liver weight, both absolute and relative, was observed with all male groups and in females in the 500 and 1500 mg/kg/day groups.

Liver weight										
	Dose									
	Males					Females				
	0	50	150	500	1500 ^a	0	50	150	500	1500
Absolute	-	↑11.9%	↑11.4%	↑21.2%	↑78.7%	-	↑1.5%	↑5.4%	↑13.6%	↑61.3%
Relative	-	↑6.0%	↑10.1%	↑19.0%	↑77.7%	-	0	↑5.0%	↑18.1%	↑63.9%

^a, only 2/5 males survived study
statistical analysis not performed

Histopathology: Adequate Battery: yes (), no (X);

Peer review: yes (), no (X)

Only tissues examined were the liver, adrenals, thyroids and ovaries.

Liver: Hepatocellular hypertrophy in both males and females at 500 and 1500 mg/kg/day dose levels.

Ovaries: Vacuolation of the interstitial cells observed with the 1500 mg/kg dose level.

Toxicokinetics:

Table 1 Toxicokinetic parameters for TAK-375 in mice

Dose (mg/kg/day)	No. of Dosing	Male			Female			Total		
		T _{max} (h)	C _{max} (µg/mL)	AUC _{0-24h} ^a (µg·h/mL)	T _{max} (h)	C _{max} (µg/mL)	AUC _{0-24h} ^a (µg·h/mL)	T _{max} (h)	C _{max} (µg/mL)	AUC _{0-24h} (µg·h/mL)
50	1st	0.5	2.56	2.0	0.5	4.08	3.6	0.5	3.32	2.8
	29th	0.5	1.85	1.6	0.5	1.34	0.9	0.5	1.60	1.3
150	1st	0.5	13.96	25.6	0.5	14.30	18.7	0.5	14.13	22.2
	29th	0.5	6.05	7.1	0.5	5.38	6.8	0.5	5.72	7.0
500	1st	0.5	35.56	80.9	0.5	31.12	85.3	0.5	33.34	83.1
	29th	1.0	5.10	35.1	1.0	3.76	24.4	1.0	4.43	30.3
1500	1st ¹⁾	1.0	91.06	613.6	1.0	48.43	680.8	1.0	69.75	647.2
	29th ²⁾	0.5	5.13	51.4	0.5	6.87	28.5	0.5	6.18	40.0

Sampling time point: n=3.

^a Calculated from mean plasma concentrations.

1) male and female: 6h, 24h (n=2)

2) male: all sampling time points (n=2); female: Pre, 24h (n=2)

T_{max} after oral administration appears to be 0.5 – 1 hr. No significant gender difference is revealed although a significant reduction in exposure with repeated drug administration is observed in both genders and at all dose levels indicative of probable hepatic enzyme induction.

Summary & Conclusions

Mice administered TAK-375 at doses of 50, 150, 500 or 1500 mg/kg/day by oral gavage for 4 weeks when compared with control animals administered vehicle (0.5 w/v% MC) demonstrated several dose-related toxicities. Of particular toxicologic interest are the **clinical signs of prone position, lying on one side, bradypnea and decreased activity** that were observed at ≥ 500 mg/kg/day dose level, especially in males. It is believed these clinical signs are related to the **mortality** observed in 3/5 males in the 1500 mg/kg/day dose level. No mortality was observed in the main study female group though 6/29 females in the toxicokinetic satellite study at this dose were found dead. It is not clear why there would be a difference between main study and satellite females. Other toxicologic alterations included **increases in cholesterol and alanine aminotransferase (ALT)** observed at 1500 mg/kg/day and **vacuolization of interstitial cells of the ovary** at 1500 mg/kg/day. The hepatic changes observed in the study (enlarged liver, increased liver weight, hepatocellular hypertrophy) is likely the result of an adaptive induction of hepatic metabolizing enzymes, as reflected in the decreased exposure with repeated administration observed at all dose levels, and is therefore not considered to be toxicologically significant. The NOAEL associated with this 4-week oral toxicity study is considered to be 150 mg/kg/day by both Sponsor and this reviewer. The TAK-375 levels associated with this dose on the final day of administration are the following: C_{max} = 5.72 µg/mL; AUC_{0-24 hr} = 7.0 µg·h/mL. The MTD from this study by this reviewer's assessment is 500 mg/kg/day.

Study title: Thirteen-Week Oral Gavage Range-Finding Toxicity Study of TAK-375 in Mice**Key study findings:**

- Mortality was observed in a single male at a dose of 1000 mg/kg/day and was considered by both Sponsor and this reviewer as treatment-related.
- Clinical signs included decreased spontaneous movement and oligopnea occurring with doses ≥ 300 mg/kg/day in males and at a dose of 1000 mg/kg/day in females. Tolerance developed to these effects by Study Day 3 (SD3).
- Body weight and body weight gain were dose-dependently reduced but did not achieve statistical significance at any dose level. Maximal body weight gain suppression was 17% in males and 8% in females at a dose of 1000 mg/kg/day relative to vehicle controls.
- Mild but statistically significant alterations of uncertain toxicologic significance was observed in both male and female mice, primarily at the 1000 mg/kg/day level though some evidence of a mildly increased Mean Cell Hemoglobin and increased platelet count was noted in males and females, respectively at a dose of 300 mg/kg/day. All values with the exception of the increase in platelets in females at the 300 and 1000 mg/kg/day level were at or below a 5% change compared to control animals.
- Changes in clinical chemistry were observed in both males and females at doses of ≥ 300 mg/kg/day. Albumin was decreased significantly in both males and females at doses ≥ 300 mg/kg/day. Mild to moderate increases in AST and ALT were noted in both sexes at the 1000 mg/kg/day dose level. Female mice exhibited a decrease in total protein and BUN with doses of ≥ 300 mg/kg/day that were not observed in male mice at any dose level.
- Gross pathology noted only enlarged (males and females) and darkened (females only) livers with the 1000 mg/kg/day dose level. Liver weight was significantly increased in both males and females at doses ≥ 300 mg/kg/day. Reductions in kidney weight were observed only in males and only at the 1000 mg/kg/day dose level.
- Histopathologic examination revealed effects in the liver consistent with increased enzyme induction (i.e. centrilobular hepatocellular hypertrophy) in both males and females with doses ≥ 300 mg/kg/day and increasing in severity with increasing dose. Vacuolization of the adrenal gland in the zona fasciculata was noted in both males and females although at doses of 1000 mg/kg/day though this appeared slightly differently depending on gender (fine vacuoles in males, large vacuoles in females). Females also showed some hypertrophy of the zona fasciculata at the 1000 mg/kg/day level as well.
- Evidence of increased hepatic enzyme metabolizing activity was supported by demonstration of increased activity of aminopyrine N-demethylase in both males and females with TAK-375 doses ≥ 100 mg/kg/day.
- Toxicokinetic analysis indicated males have a higher exposure to TAK-375 parent and the active metabolite M-II than females though significant reduction in exposure is observed with repeated drug administration (as much as 10-fold reduction, consistent with enzyme induction) in both genders. M-II exposure in males was relatively similar to exposure to parent while in females M-II exposure was approximately half that of the

- parent. Females demonstrate a greater exposure to the inactive metabolite M-III than males at the same dose level.
- The NOAEL identified by this reviewer in this study is **100 mg/kg/day** for both males and females due to clinical signs of decreased spontaneous movement and oligopnea in males as well as mild changes in hematologic and clinical chemistry parameters at a dose of 300 mg/kg/day. The Sponsor considers the mild hematologic changes to be of little toxicologic significance and considered the NOAEL to be 300 mg/kg/day. Although this reviewer believes that although the changes were small they were fairly widespread within the panel and indicate perturbation of hepatic function which becomes more severe with increases in dose. Additionally, the clinical signs demonstrated in males at 300 mg/kg/day is considered adverse.

Plasma concentrations of TAK-375 and metabolites at the NOAEL and Clinical Exposure Margin for Humans[†]

TAK-375		M-I		M-II		M-III		M-IV	
AUC ₀₋₂₄ (ng·h/mL)	Exposure Margin	AUC ₀₋₂₄ (ng·h/mL)	Exposure Margin	AUC ₀₋₂₄ (ng·h/mL)	Exposure Margin	AUC ₀₋₂₄ (ng·h/mL)	Exposure Margin	AUC ₀₋₂₄ (ng·h/mL)	Exposure Margin
8681	924	6663	833	7143	30	28885	824	9461	166

[†], using Sponsor's estimated values of AUC₀₋₂₄hr for TAK-375 (9.4 ng·h/mL), M-I (8 ng·h/mL), M-II (241.3 ng·h/mL), M-III (3.5 ng·h/mL), and M-IV (57 ng·h/mL)

Study no.: M-11-00178

Module: 4.2.3.4.1 Long-term Studies

Conducting laboratory and location: ☐

☐

Date of study initiation: December 25, 1998

GLP compliance: Yes, Japan

QA report: Yes

Drug, lot #, and % purity: TAK-375, lot# M375-010, purity ☐ ☐ COA, stability and homogeneity information supplied as attached data.

Methods

Doses: 0 (0.5 w/v% methylcellulose) or TAK-375 30, 100, 300 or 1000 mg/kg/day in 0.5 w/v% methylcellulose

Species/strain: — B6C3F₁ mice; ☐ 1

Number/sex/group or time point (main study): 12 mice/sex/group

Route, formulation, volume, and infusion rate: Oral gavage of TAK-375 in 0.5 w/v% methylcellulose administered as 10 mL/kg

Satellite groups used for toxicokinetics or recovery: 43 mice/sex/group for toxicokinetic analysis

Age: 6 weeks at study initiation

Weight: 22.0 – 26.5 g (males); 17.5 – 21.8 g (females)

Sampling times: Toxicokinetic samples obtained after the first dose and in Week 13 (days 89 and 90). Six or 7 timepoints were assessed: Pre-dose only in Week 13, 0.25, 0.5, 1, 3, 6, and 24 hr after dosing on both study days.

Unique study design or methodology (if any):

Test group	Dose Level (mg/kg)	Concentration of Test Suspension (mg/mL)	Dose Volume (mL/kg)	Sex	Main Group		Satellite Group	
					No. of Animals	Animal Number	No. of Animals	Animal Number
Control	0	0	10	M	12	1001 - 1012	-	-
				F	12	1101 - 1112	-	-
Low	30	3	10	M	12	2001 - 2012	43	2013 - 2055
				F	12	2101 - 2112	43	2113 - 2155
Middle	100	10	10	M	12	3001 - 3012	43	3013 - 3055
				F	12	3101 - 3112	43	3113 - 3155
High	300	30	10	M	12	4001 - 4012	43	4013 - 4055
				F	12	4101 - 4112	43	4113 - 4155
Highest	1000	100	10	M	12	5001 - 5012	43	5013 - 5055
				F	12	5101 - 5112	43	5113 - 5155

Best Possible Copy

Note: Study Day 1 (SD1) is the first day of drug administration.

Observation Times & Results

Mortality: (Assessed 3 times everyday except Saturday and Holidays on which animals were observed twice daily: prior to dose administration, immediately afterward and 1 hr after drug administration). One male in the 1000 mg/kg/day group died on SD2 demonstrating prone/lateral positioning, shallow and uneven respiration and evidence of hypothermia. No abnormalities noted on necroscopic exam. Histopathology indicated a slightly increased cellular debris in the bone marrow, mild dilation of the pelvis of the kidney, and mild atrophy of white pulp in spleen. This was considered both by the Sponsor and this reviewer to be a drug-related death.

Clinical signs: (Assessed 3 times everyday except Saturday and Holidays on which animals were observed twice daily: prior to dose administration, immediately afterward and 1 hr after drug administration).

In males, no clinical observations were notable with the 30 or 100 mg/kg/day dose level. The 300 mg/kg/day dose level was associated with decreased spontaneous movement on SD1 in 6/12 mice immediately after dosing and at 1 hr post-dose but was not observed in any animal administered this dose on subsequent days. The 1000 mg/kg/day dose was associated with decreased spontaneous movement in 12/12 mice while prone/lateral position and oligpnea was observed in 2/12 animals immediately after dosing on SD1. At 1 hr after dosing the decreased spontaneous movement was still observed in nearly half the animals while prone/lateral position was observed in the other half of animals while 2 animals were considered normal. On SD2, decreased spontaneous movement was noted in 4 animals upon drug administration, one of which demonstrated prone/lateral position and oligopnea. At 1 hr after dosing this was observed in 2 animals. No clinical signs were observed from SD3 onward to the end of study which suggests a tolerance develops.

In females, no abnormal clinical signs were observed with administration of 30, 100 or 300 mg/kg/day TAK-375. Decreased spontaneous movement, as in the males, was observed in the majority of females immediately after dosing on SD1. Decreased movement and prone/lateral position was observed in 3 animals and Oligopnea was seen in 1 animal at 1 hr after the initial administration of the 1000 mg/kg/day dose level. SD2 administration of TAK-375 was associated with decreased spontaneous movement in 2/12 mice but after SD2, no clinical signs were associated with TAK-375 treatment at any dose level.

Body weights: (Animals weighed twice in the first week: on SD1 prior to dosing and on SD7. Animals weighed once a week for the duration of the study after the first week). Body weight was generally unaffected by TAK-375 administration, though females of the 300 mg/kg/day group showed a decreased body weight (↓6%; $p < 0.001$) on SD70 (Week 10). Body weight at this dose level was not significantly different on any other observation, notably SD63 and SD77, and was not different in the 1000 mg/kg/day group at any observation so the Sponsor believes these observations to be a data artifact. Nevertheless, in the males, there appears to be a dose-related reduction in weight when assessed both as an absolute and as a proportion of body weight gain.

Body Weight/Body Weight Gain with Administration of TAK-375										
	Males (dose mg/kg/day)					Females (dose mg/kg/day)				
	0	30	100	300	1000	0	30	100	300	1000
Body Weight (% control)	-	↓1	↓2.6	↓3.6	↓5.2	-	-	↑1.0	↓3.3	↓2.2
Body Weight Gain (% control)	-	↑1.5	↓7.6	↓13.6	↓16.7	-	-	↑2.6	↓7.9	↓7.9
All overall group values are nonsignificant ($p > 0.05$)										

Food consumption: (Food consumption from day before first administration was recorded and on SD7 was calculated by dividing 6 days cumulative consumption. Each week's consumption was divided by 7 days to derive the daily consumption). Single occurrences of significantly increased food consumption was found in both 300 (SD21) and 1000 mg/kg/day (SD42) males and in females at 1000 mg/kg/day (SD21). The Sponsor considers the sporadic effects on food consumption to be incidental though this reviewer notes in both sexes the food consumption value was nearly always ~ 5% higher in the 1000 mg/kg/day group than in controls.

Ophthalmoscopy: Not done

EKG: Not done

Hematology: (Standard parameters obtained at time of necropsy from inferior vena cava after overnight fast. Parameters consist of RBC, Hb, Ht, MCV, MCH, MCHC, reticulocyte ratio, platelets and WBC without differential).

Male mice administered TAK-375 at doses of ≥ 300 mg/kg/day demonstrated an increase in MCH while males in the 1000 mg/kg/day group demonstrated decreased RBC and increased MCV.

Female mice at a dose ≥ 300 mg/kg/day had significantly increased platelets while those at a dose of 1000 mg/kg/day also had an increase in MCV and MCH. See table below.

Hematology								
Dose (mg/kg/day)	% Change vs. Control							
	Males				Females			
	30	100	300	1000	30	100	300	1000
RBC	---	---	---	↓3%*	---	---	---	---

MCV	---	---	---	↑3%**	---	---	---	↑1%*
MCH	---	---	↑2%*	↑3%**	---	---	---	↑2%**
Platelets	---	---	---	↑5%	↑3%	---	↑6%*	↑10%**
---, non-significant change or not notable								
*, p< 0.05; **, p<0.01								

Clinical chemistry: (Obtained at the time of necropsy from the inferior vena cava after overnight fast. Parameters assessed included AST, ALT, AIP, total cholesterol, glucose, BUN, Creatinine, total protein, albumin, A/G ratio).

In males, all TAK-375 groups demonstrated a rise in AST which was only significant at 1000 mg/kg/day. At the 300 mg/kg/day dose, significant decreases in albumin and total cholesterol were noted while all other changes did not reach statistical significance. Males administered 1000 mg/kg/day demonstrated the increased AST, an increase in ALT and decreases in albumin, the A/G ratio and total cholesterol, though the latter reduction was not dose-related. Creatinine was significantly elevated at this dose level.

Females treated with TAK-375 also demonstrated an apparent dose-dependent rise in AST significant only at the 1000 mg/kg/day level. No significant changes were observed with the 30 or 100 mg/kg/day dose level but at ≥300 mg/kg/day a mild, though significant decrease in total protein, albumin and total cholesterol was noted. Blood urea nitrogen and creatinine were slightly elevated suggestive perhaps of mildly impaired renal function, though BUN increase was not dose-related. In the female mice treated with 1000 mg/kg/day TAK-375 AST and ALT were increased significantly as was creatinine.

Clinical Chemistry								
Dose (mg/kg/day)	% Change vs. Control							
	Males				Females			
	30	100	300	1000	30	100	300	1000
AST	↑17%	↑7%	↑17%	↑21%*	↑8%	↑25%	↑42%	↑63%*
ALT	---	---	↑13%	↑100%**	---	---	↑21%	↑96%**
Total Protein	---	---	---	---	---	---	↓5%*	↓5%**
Albumin	---	---	↓3%*	↓7%**	---	---	↓6%**	↓10%**
A/G ratio	---	---	---	↓9%*	---	---	---	↓9%*
Total chol.	---	---	↓13%*	↓7%*	---	---	↓11%*	↑21%**
BUN	---	---	---	---	---	---	↑32%*	↑18%*
Creatinine	---	---	↑5%	↑29%*	---	---	↑8%	↑14%*
---, not statistically significant nor notable, *p<0.05, **p<0.01								

Urinalysis: Not done

Gross pathology: (External appearance of organs and tissues in the abdominal, thoracic and cephalic cavities were examined and abnormalities recorded. Animals found dead were promptly necropsied).

Liver: Liver was enlarged in 11/11 males and 5/12 females with discoloration (dark) noted in all females at the 1000 mg/kg/day.

No other findings on necropsy were considered to be related to drug treatment.

Organ weights: (Organs weighed included brain, thymus, heart lungs (w/bronchus), liver (w/gallbladder), spleen, kidneys (both right and left) and testes (both right and left). Organs from mice found dead were weighed but were not included in the data analysis).

Absolute and relative liver weight was increased in both males and females significantly with dose levels of ≥ 300 mg/kg/day. A significant decrease in absolute and relative kidney weight was only observed in males at the 1000 mg/kg/day level. All other organ weights were unaffected by TAK-375 treatment.

Organ Weights[†]										
(% Change from Control)										
	Dose (mg/kg/day)									
	Males					Females				
	0	30	100	300	1000	0	30	100	300	1000
Liver	-	↑2%	↑6%	↑18%**	↑51%**	-	↑4%	↑7%	↑11%**	↑43%**
Kidney	-	↑2%	↓2%	↓5%	↓11%**	-	---	---	---	---

[†], body weight was not significantly affected by treatment and relative organ weight changes are similar to absolute and are not shown.
 **p<0.01; ---, no notable change

Histopathology: Adequate Battery: yes (X), no ();

Peer review: yes (), no (), uncertain

(Organs and tissues were fixed in 10% formalin in PBS except eyeballs, Harderian gland and testes which were fixed in organ specific fixatives prior to embedding in paraffin. Sectioning and microscopic examination of organs and tissues were only conducted in the control and high dose groups with the exception of the liver and adrenals in both males and females and the kidneys in males in which all animals in all dose groups were examined. Paired organs indicated by asterisks (*) were also examined. The following list is reproduced from the Sponsor's submission.

cerebrum, cerebellum, spinal cord (thoracic), sciatic nerve, eyeballs*, Harderian glands*, pituitary, thyroids (including parathyroids)*, adrenals*, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, thoracic aorta, trachea, lungs (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular glands*, sublingual glands*, liver, gallbladder, pancreas, kidneys*, urinary bladder, testes*, epididymides*, prostate, seminal vesicles*, ovaries*, uterus (both horn), vagina, mammary gland (females only), sternum (including bone marrow), femur (including bone marrow), skeletal muscle (femoral muscle), skin (inguinal)

Microscopic analysis of selected tissues revealed the following:

Adrenal gland: Males demonstrated fine vacuolization of the adrenal gland, characterized as slight in nature, and only in the 1000 mg/kg/day dose level. Females also demonstrated histopathologic findings in the adrenal gland at the 1000 mg/kg/day but these were characterized as hypertrophy and vacuolization of the zona fasciculata, both findings considered slight in severity.

Liver: Both males and females were noted to exhibit centrilobular hepatocellular hypertrophy with doses ≥ 300 mg/kg/day and to progress primarily from slight in severity at 300 mg/kg/day to mild at the 1000 mg/kg/day dose level in both genders.

Microscopic Findings from 13-Week Oral Dose Range-Finding Toxicity Study in the Mouse										
ORGAN SYSTEM [†]	Males					Females				
	Dose Administered (mg/kg/day)					Dose Administered (mg/kg/day)				
	0	30	100	300	1000	0	30	100	300	1000
# Examined	12	12	12	12	12	12	12	12	12	12
Adrenal										
Hypertrophy, zona fasciculata										
Slight	0	0	0	0	0	0	0	0	0	4
Vacuole, large, zona fasciculata										
Slight	0	0	0	0	0	0	0	0	0	5
Vacuole, fine, zona fasciculata										
Slight	0	0	0	0	4	0	0	0	0	0
Liver										
Centrilobular hypertrophy										
Slight	0	0	0	6	11	0	0	0	4	12
Mild				6	1				4	2
				0	10				0	10

[†], All other tissues were judged normal or did not show treatment-related effects

Determination of Hepatic Metabolizing Enzyme Activity: (Sections of liver were taken at the time of necropsy from 5 animals/sex/group for the determination of aminopyrine N-demethylase and aniline hydroxylase).

Liver samples from both male and female mice demonstrated an increase in aminopyrine N-demethylase activity that was dose-dependent and achieved statistical significance at ≥ 100 mg/kg/day dose levels. The activity of aniline hydroxylase was statistically significant in females at the 300 mg/kg/day level but a clear dose-dependent effect was not observed.

Activity of Selected Drug Metabolizing Enzymes (% Change from Control)										
	Dose (mg/kg/day)									
	Males					Females				
	0	30	100	300	1000	0	30	100	300	1000
Aminopyrine N-demethylase	-	↑11%	↑23%*	↑24%*	↑48%*	-	↑16%	↑35%**	↑57%**	↑62%**
Aniline hydroxylase	-	↑1%	↑1%	↑3%	↑7%	-	↑5%	↑10%	↑16%*	↑11%

*p<0.05, **p<0.01 vs. control values

Toxicokinetics: (Blood samples were collected from animals on two days – once after the first dose and once after the 89th or 90th dose in Week 13 at 6 different timepoints on SD1, 7-timepoints on SD89/90: pre-dose (SD89/90 only), 0.25, 0.5, 1, 3, 6, and 24 hr after drug administration. Blood was collected from the inferior vena cava at the time of sacrifice. The plasma concentration of the parent compound, TAK-375 as well as the concentrations of

metabolites M-I through M-IV and including the active metabolite M-II were determined by $\square$ } Additionally, the optical isomer ratios of M-II (2S, 8S to 2R, 8S) were determined at 0.25 and 1 hr after dosing in the 100 mg/kg/day group and 0.25, 1, and 6 hr after dosing in the 1000 mg/kg/day group using $\square$ } methods).

Males: TAK-375 plasma concentrations

Dose mg/kg	Plasma concentration (µg/mL)										C _{max} (µg/mL)	T _{max} (hr)	AUC 0-24hr (µg hr/mL)
	Animal number	0.25hr	Animal number	0.5hr	Animal number	1hr	Animal number	2hr	Animal number	6hr			
30	3036	1.335	3041	0.693	3044	ND	3047	ND	3050	ND	3053	ND	
	3038	1.632	3043	0.448	3045	0.978	3048	ND	3051	ND	3054	ND	
	3040	1.807	3043	0.417	3046	0.928	3049	ND	3052	ND	3055	ND	
	Mean	1.591		0.519		0.632		0		0			
S.D.	0.230		0.151		0.028								
100	3036	16.823	3041	10.851	3044	2.820	3047	ND	3050	ND	3053	ND	
	3038	9.770	3043	0.541	3045	3.278	3048	0.147	3051	ND	3054	ND	
	3040	17.171	3043	7.637	3046	0.928	3049	ND	3052	ND	3055	ND	
	Mean	14.891		5.750		3.078		0.049		0			
S.D.	4.134		1.211		0.974		0.063						
300	4036	21.315	4041	21.761	4044	15.143	4047	6.751	4050	12.657	4053	ND	
	4038	47.280	4043	21.706	4045	29.061	4048	6.167	4051	12.086	4054	ND	
	4040	27.685	4043	29.892	4046	15.820	4049	6.820	4052	6.491	4055	ND	
	Mean	32.127		21.453		19.381		7.179		11.078			
S.D.	13.540		6.487		5.752		1.281		2.229				
1000	5036	56.631	5041	47.333	5044	25.957	5047	28.725	5050	29.400	5053	1.442	
	5038	80.711	5043	28.933	5045	20.527	5048	56.955	5051	29.470	5054	2.089	
	5040	86.758	5043	26.992	5046	83.927	5049	41.843	5052	6.444	5055	2.488	
	Mean	62.037		36.619		48.817		39.774		28.438		1.973	
S.D.	4.321		10.358		32.240		18.187		10.047		0.490		

ND : Not detectable (µg/mL) was treated as zero(0) to calculate the Mean±S.D.

Females: TAK-375 plasma concentrations

Dose mg/kg	Plasma concentration (µg/mL)										C _{max} (µg/mL)	T _{max} (hr)	AUC 0-24hr (µg hr/mL)
	Animal number	0.25hr	Animal number	0.5hr	Animal number	1hr	Animal number	2hr	Animal number	6hr			
30	3138	0.919	3141	0.430	3144	0.976	3147	ND	3150	ND	3153	ND	
	3139	1.314	3142	0.483	3145	0.442	3148	ND	3151	ND	3154	ND	
	3140	1.513	3143	0.354	3146	0.046	3149	ND	3152	ND	3155	ND	
	Mean	1.252		0.418		0.655		0		0			
S.D.	0.307		0.057		0.029								
100	3138	12.360	3141	5.350	3144	3.588	3147	0.056	3150	ND	3153	ND	
	3139	14.213	3142	9.377	3145	3.229	3148	0.173	3151	ND	3154	ND	
	3140	8.428	3143	10.824	3146	1.895	3149	0.037	3152	ND	3155	ND	
	Mean	11.700		8.546		2.907		0.080		0			
S.D.	2.997		2.486		0.852		0.074						
300	4138	23.738	4141	22.156	4144	18.220	4147	2.113	4150	2.202	4153	ND	
	4139	23.895	4142	24.581	4145	18.904	4148	0.878	4151	1.388	4154	ND	
	4140	21.634	4143	25.844	4146	18.789	4149	3.878	4152	1.092	4155	ND	
	Mean	23.486		23.454		17.898		4.100		1.601			
S.D.	2.892		1.214		1.448		2.441		0.575				
1000	5138	52.028	5141	29.158	5144	48.411	5147	6.942	5150	5.784	5153	ND	
	5139	42.829	5142	97.299	5145	34.818	5148	22.086	5151	5.084	5154	ND	
	5140	47.289	5143	38.054	5146	38.136	5149	9.099	5152	7.803	5155	ND	
	Mean	47.316		55.026		40.454		12.698		6.170			
S.D.	4.780		38.908		7.688		8.188		1.342				

ND : Not detectable (µg/mL) was treated as zero(0) to calculate the Mean±S.D.

TAK-375 metabolite concentrations in Week 13

Dose (mg/kg/day)	Analyte	Male (n=3)			Female (n=3)			Total (n=6)		
		Tmax (h)	Cmax (ng/mL)	AUC ₀₋₂₄ * (ng·h/mL)	Tmax (h)	Cmax (ng/mL)	AUC ₀₋₂₄ * (ng·h/mL)	Tmax (h)	Cmax (ng/mL)	AUC ₀₋₂₄ * (ng·h/mL)
30	TAK-375	0.3	4048	1579	0.3	2370	996	0.3	3209	1288
	M-I	0.3	2533	1538	0.3	5410	2696	0.3	3971	2117
	M-II	0.3	2000	1294	0.3	823	455	0.3	1411	875
	M-III	0.3	12247	7351	0.3	8805	5269	0.3	10526	6310
	M-IV	0.5	3092	3729	0.5	1570	1464	0.5	2331	2597
100	TAK-375	0.3	9486	9672	0.5	6388	7690	0.3	7662	8681
	M-I	1.0	2790	5839	0.3	7362	7486	0.3	5044	6663
	M-II	1.0	4776	8521	1.0	3254	3764	1.0	4015	7143
	M-III	1.0	17089	30841	1.0	13815	26929	1.0	15452	28885
	M-IV	1.0	4279	11601	1.0	4004	7320	1.0	4142	9461
300	TAK-375	0.5	9682	46311	0.3	11337	18098	0.3	10123	32205
	M-I	0.5	3979	17631	0.3	14598	24883	0.3	9208	21258
	M-II	1.0	3563	39136	1.0	2167	7387	1.0	2865	33262
	M-III	0.5	11571	78969	0.5	16558	55718	0.5	14063	67344
	M-IV	1.0	2126	26930	1.0	2173	16318	1.0	2150	21624
1000	TAK-375	1.0	12537	43057	1.0	8332	29214	1.0	10433	36136
	M-I	1.0	7140	33372	0.3	13278	59924	0.3	9628	46648
	M-II	1.0	3056	28312	1.0	1852	11884	1.0	2454	20098
	M-III	1.0	9214	56960	1.0	11543	80106	1.0	10379	68333
	M-IV	3.0	1782	19662	3.0	1562	19164	3.0	1672	19413

* Calculated from mean plasma concentrations.

	Dose (mg/kg/day)	Males		Females	
		C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·h/mL)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·h/mL)
TAK-375	30	4,048	1,579	2,370	996
	100	9,486	9,672	6,388	7,690
	300	9,682	46,311	11,337	18,098
	1000	12,537	43,057	8,332	29,214
M-II	30	2,000	1,294	823	455
	100	4,776	8,521	3,254	5,764
	300	3,563	39,136	2,167	7,387
	1000	3,056	28,312	1,852	11,884

TAK-375: Exposure appeared to plateau in male mice at 300 mg/kg/day while female exposure increased in the 1000 mg/kg/day dose level. Males generally had an exposure which was 25-155% higher than females at the same dose level. Exposure for both males and females appeared to decrease significantly (up to 10-fold) with repeated administration of TAK-375 during the 13-week course of the study. T_{max} was ~ 0.3 – 1 hr generally.

M-II: Exposure to the active metabolite M-II was approximately the same as to TAK-375 in male mice while only half that of TAK-375 in the female mice. Exposure increased with increasing dose throughout the dose range except in male mice where the 1000 mg/kg/day dose produced an exposure that was lower than observed at the 300 mg/kg/day level. T_{max} was ~ 0.3 – 1 hr generally.

Isomer ratios for M-II: For both males and females and regardless of dose, the 2*S*, 8*S* isomer of M-II predominates, circulating at approximately 15-fold higher concentrations than the 2*R*, 8*S* isomer.

2.6.6.4 Genetic toxicology

The following studies were reviewed by Aisar Atrakchi, Ph.D. in the Division of Neuropharmacologic Products (HFD-120) and are reproduced from the review of the original IND submission and serial submission #013.

Study Title: Bacterial Reverse Gene Mutation
 Study No: M-11-13
 Volume # 7; page# 1
 Conducting Laboratory: [redacted] J
 Date of Study Initiation/completion: Oct 1997/Jan 1998
 GLP Compliance: Yes/Japanese
 QA- Reports Yes (x) No ():
 Drug Lot Number: M375-002
 Study Endpoint: revertant colony count

Methodology:

- Bacterial Strains: TA100, TA98, TA1535, TA1537, E.coli WP2uvrA
- Dose Selection Criteria: dose range finder
- Basis of dose selection: cytotoxicity
- Range finding studies: concentrations tested in +&-S9: 51.2, 128, 320, 800, 2000, and 5000ug/ml.
- Test Agent Stability: solid material: the test sub was stable for — at 60&40C
 in solution: test sub was stable in the 1st and 2nd solutions of the Japanese Pharmacopoeia for — r at 37C.
- Solubility: TAK375 is freely soluble in DMSO and ethanol; low solubility in water at 200ug/ml pH1-9 at 37C.
- Metabolic Activation System: Aroclor induced rat liver.
- Vehicle & Solvent control: DMSO
- Positive Controls: TA100, TA98, WP2uvrA AF-2
 TA1535 NaN3
 TA1537 9-AA
- Incubation and sampling times: preincubation for 20min at 37C; after agar overlay, incubation was for 48hr at 37C.
- Doses used in definitive study: 156, 313, 625, 1250, 2500, 5000ug/plate; visible ppt was seen at the 5000ug/plate concentration at end of exposure period.
- Study design: dose range finder and main assay; both in -&-S9.

- Analysis:
 - plates: triplicate plates for the solvent, 2 plates/dose for each strain with the drug and the positive controls.
 - Counting method: automated colony analyzer
 - Cytotoxic endpoints: diminished background lawn.
- Genetic toxicity endpoints/results: increase in number of revertant colonies (see criteria for positive finding below).
 - Statistical methods: not used.
- Criteria for Positive Results: results were positive when the number of revertant colonies was increased more than 2x that in concurrent solvent control and a dose dependent relationship or reproducibility was seen.

Results:

- Study Validity: valid
- Study Outcome: **Negative**

Summary:

TAK375 tested in - and +S9 in 5 Salmonella and E.coli tester strains for its potential to induce genetic mutation by increasing the number of revertant colonies at least 2x over those seen in the solvent control plates. The number of revertant colonies in TAK375-treated plates was comparable to those of the control at all concentrations tested and upto 5000ug/plate. A clear increase was seen in the positive control plates. TAK375 was negative in both the dose range finder and the main assay.

Study Title: In vitro Mammalian Cell Gene Mutation/MLP L5178Y/TK+/-
Study No: M-11-19
Volume # 7; page# 44
Conducting Laboratory:

J

Date of Study Initiation/completion: Oct 1997/Apr 1998

GLP Compliance: Yes: FDA & Japanese

QA- Reports Yes (x) No ():

Drug Lot Number: M375-002

Study Endpoint: reduction in suspension growth for cytotoxicity and increase in mutant frequency in the main assay.

Methodology:

- Cell line: L5178Y/TK-/+ mouse lymphoma cells.
- Dose Selection Criteria: dose range finder
- Basis of dose selection: suspension growth relative to that of the concurrent solvent control.
- Range finding studies: concentrations tested in - & -S9: 0.5-2500ug/ml (9 concentrations).

- Test Agent Stability & Solubility: responsibility of sponsor, drug substance of this lot was shown to be stable and soluble in DMSO (400mg/ml).
- Metabolic Activation System: Aroclor 1254-induced rat liver S9.
- Vehicle control: DMSO
- Positive Controls: MMS at 10&20ug/ml in -S9 and DMBA (dimethylbenzanthracene) 2.5&4ug/ml in +S9.
- Incubation and sampling times: method done according to that by Clive and Spector (1975). Total of at least 8 concentrations were tested in duplicates. Exposure of cells to the drug and positive and solvent controls was for 4hr at 37C.
- Doses used in definitive study: in +S9 50, 100, 150, 200, 300ug/ml and in -S9: 100, 200, 300, 400, 500ug/ml.
- Study design: dose range finder and main assay, both in -&+S9.
 - Analysis:
 - plates: triplicate plates for the solvent, positive controls, and each drug dose.
 - Counting method: electronic cell counter and automated colony analyzer.
 - Cytotoxic endpoints: reduction in suspension growth after treatment.
 - Genetic toxicity endpoints/results: increase in forward mutation reflected by increase in mutant frequency over the solvent control values.
 - Statistical methods: not used.
 - Criteria for Positive Results: positive response if there is a concentration related increase in mutant frequency and 1 or more concentrations with $\geq 10\%$ total growth showing MF of ≥ 100 mutants per 10^6 clonable cells over the background level.

Results:

- Study Validity: valid
- Study Outcome: **Negative**

Summary:

The preliminary cytotoxicity assay was done in - and +S9 using the drug, the solvent control DMSO and positive controls: drug concentrations ranged between 0.5-2500ug/ml. A visible ppt was seen at ≥ 1500 ug/ml in both -/+S9 and zero suspension growth was seen at 500ug/ml relative to the solvent control. Therefore, doses selected for the main assay ranged from 10-500ug/ml for both - and +S9. In the main assay, a ppt was seen at 500ug/ml. In -S9, suspension growth ranged between 18-97% of the control and that in +S9 ranged between 10-101%. Total growth ranged from 16-81% in -S9 at concentrations 100-500ug/ml and from 12-105% in -S9 at 100-400ug/ml. Based on these findings, an independent repeat assay was done at concentrations between 50-600ug/ml for the -S9 and 10-500ug/ml in +S9. Again, a ppt was seen at ≥ 500 ug/ml. In the -S9, cultures that were cloned were those at concentrations of 100, 200, 300, 400, and 500ug/ml and produced suspension growth between 9-81% and total growth of 6-91%. In the +S9, cultures cloned were those at concentrations of 50, 100, 150, 200, 300ug/ml and produced suspension growth between 16-107% of control and total growth between 15-128%. At no concentration tested in any of the assays was there an increase in MF either in presence or absence of S9. Therefore, TAK375 was **not mutagenic** in the MLP/TK locus assay. TFT colony sizing was also done in this assay for the positive and negative controls and the expected responses were seen (i.e. increase in small colony size for the positive controls).

Study Title: In vitro Chromosomal Aberration assay in CHL cells
Study No: M-11-17
Volume # 7; page# 95
Conducting Laboratory: []
Date of Study Initiation/completion: Oct, 1997/Feb 1998
GLP Compliance: Yes/Japanese
QA- Reports Yes (x) No ():
Drug Lot Number: M375-002
Study Endpoint: increase in chromosomal aberrations (structural and/or numerical) above the concurrent solvent control.

Methodology:

- Cell line: Chinese hamster lung cells.
- Dose Selection Criteria: dose range finder.
- Basis of dose selection: cytotoxicity based on mitotic index (MI) relative to that of the solvent control.
- Range finding studies: concentrations tested in +&-S9: 8 concentrations ranged between 26-936ug/ml for the direct methods (24&48hrs) and for the activation method in -S9 from 72.8-1560ug/ml and in -S9 from 202-2600ug/ml.
- Test Agent Stability & Solubility: solid drug substance stability: stable for — at 60&40C. stability in solution: test sub stable in the 1st and 2nd solutions of the Japanese pharmacopeia for — at 37C. Test sub freely soluble in DMSO and ethanol; solubility in water is small at 200ug/ml at 37C and pH 1-9.
- Metabolic Activation System: phenobarbital-induced rat liver S9.
- Vehicle control: DMSO
- Positive Controls: in -S9: MMS at 0.05ug/ml for the 24hr and 0.025ug/ml for the 48hr test. for the +S9: CP at 12.5ug/ml.
- Incubation and sampling times: see summary section.
- Doses used in definitive study: 56.3, 113, 225, 450ug/ml for the direct assay at 24&48hr and for the activation assay in -S9: 113, 225, 450, 900ug/ml for the activation assay in +S9.
- Study design: pilot/dose range finder and mam assay, both in -&+S9: a direct method done at 24&48hr incubation times and activation method at 6-18hr incubation.
- Analysis:
 - plates: 2 wells per dose for the solvent, positive controls, and each drug dose.
 - Counting method: assessment of cell survival rate (ratio of absorption of each test gr compared to that of the solvent control), by measuring absorption of each plate with a spectrophotometer and. 50% cell growth inhibition was determined by Probit's method (ref was not provided).
 - Cytotoxic endpoints: inhibition of MI and reduction in survival.
- Genetic toxicity endpoints: results: increase in chromosomal aberrations (structural and or numerical) over the solvent control values.
 - Statistical methods: not used.
- Criteria for Positive Results: positive response if is reproducible or dose dependent

Results:

- Study Validity: questionable: see below.
- Study Outcome: Positive

Comments:

The following are comments on the conduct of the assay:

- On page#16 section 10.10 Results of cell growth inhibition and MI counting, the statement: "50% cell growth inhibition doses of 368, 178, 427, and 475ug/ml for the direct method....." it is unclear where these concentrations came from; they were not mentioned in an earlier section nor presented in any table; it is requested that the sponsor clarifies this.

- In the results section 11.3, CP was used as the positive control in -S9; it is known that this control is used in +S9. Therefore, absence of induction of any chrom abs in CP treated cultures is not surprising. The conclusion of this part of the assay is therefore, questionable since the validity of the test can not be determined in absence of a positive control.

- In the main assay - activation method/+S9 the MI as percent of control in the 113 and 225ug/ml doses was not inhibited i.e. greater than the solvent control value (107&116.9% vs. 100 in the control). At the 450ug/ml, although a ppt was seen, MI was 64% of control or inhibition of 36%. A positive response i.e. increase in chromatid breaks and exchanges was seen at the 450ug/ml in +S9 activated assay which greatly exceeded the concurrent solvent control value of 1 total aberrations (the drug at 450ug/ml showed total of 29 aberrations). Therefore, validity of the results at 113&225ug/ml is questionable.

Summary & Conclusion:

In the pilot study, a visible ppt was seen at 337ug/ml in the direct and activation assays in both presence and absence of S9. Table below from sponsor presents the MI results for the direct and activation assays:

Chromosomal Abnormalities in CHL cells treated with TBE-528 in the pilot study

Compound	Direct method					Activation method				
	Dose (ug/ml)	24 hrs		48 hrs		Dose (ug/ml)	24 hrs		48 hrs	
		Chrom. Index	Percent of control	Chrom. Index	Percent of control		Chrom. Index	Percent of control	Chrom. Index	Percent of control
TBE 528 a)	0	2.4	100.0	6.4	100.0	0	6.2	100.0	7.2	100.0
		0.2		4.7			6.6		6.4	
TBE-528	36.8	0.0	0.0	5.6	88.0	72.8	5.0	79.0	4.2	66.3
		0.2		6.4			4.2		6.4	
TBE-528	43.7	0.2	0.0	3.0	47.0	181	6.6	106.1	3.8	58.3
		0.4		3.0			3.8		5.6	
TBE-528	72.8	4.0	16.7	4.0	62.5	302	1.2	19.4	2.0	31.7
		5.0		5.4			1.4		2.8	
TBE-528	113	4.0	16.7	4.2	65.6	337.41	3.2	51.8	6.6	102.6
		4.0		6.1			4.2		7.1	
TBE-528	225	3.0	50.0	3.0	47.0	562.41	3.6	58.1	1.6	25.0
		3.0		2.2			3.6		1.4	
TBE-528	337.41	3.0	47.0	0.1	1.6	936.41	None		2.4	37.5
		0.6		0.4			3.0		3.0	
TBE-528	562.41	0.0	0.0	None		1569.41	3.2	51.5	5.6	87.5
		3.0		None			4.0		7.8	
TBE-528	936.41	0.0	0.0	None		1846.41			1.6	25.0
		0.0		None					6.6	

a) : Solvent control
 b) : Visible precipitation was observed at the end of exposure period
 (): None

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From this table, it can be seen that the MI was inhibited in the 24&48hr direct methods and upto 562ug/ml concentration in the -&+S9 activation method after which it was increased perhaps due to toxicity (ppt was seen starting at 337ug/ml). There was a dose dependent increase in chromosomal aberrations including polyploidy in the direct method (both 24&48 hrs; tables below from sponsor), and in the +S9 of the activation method at the 450ug/ml concentration (table below from sponsor).

Pilot Study:

(direct method : 24 hrs)

Compound	Dose (ug/ml)	Number of Cells	Number of cells with structural aberrations						Total (ppt) (%)	Total (-ppt) (%)	Polyploid cells (%)	Final judgement
			gpp	ctb	ctc	ctd	cse	ctf				
NEG a)	0	200	1	0	0	0	0	0	0.1	0.0	1.0	-
TAL-275	56.2	200	1	2	1	0	0	0	2.0	1.5	1.1	-
	112	200	2	5	2	0	0	0	4.5	3.5	1.2	-
	225	200	1	5	2	0	0	1	4.5	4.0	1.2	-
	650 d)	Toxic										
MC b)	0.05	200	5	30	11	0	0	1	45.0	43.0	10.0	+

ctb: Chromatid break ctc: Chromatid exchange ctd: Chromosome break cse: Chromosome exchange ctf: others
 a): Solvent control
 b): Positive control (Mitomycin C)
 c): Possible overexposure was occurred at the end of exposure period
 d): An estimate based on observation 1000 cells

Compound	Dose (ug/ml)	Number of Cells	Number of cells with structural aberrations						Total (ppt) (%)	Total (-ppt) (%)	Polyploid cells (%)	Final judgement
			gpp	ctb	ctc	ctd	cse	ctf				
NEG a)	0	200	0	0	0	0	0	0	0.5	0.5	1.0	-
TAL-275	56.2	200	1	0	1	0	0	0	2.0	2.5	1.5	-
	112	200	0	0	2	0	0	0	2.0	1.6	2.1	-
	225	200	0	0	3	0	0	0	3.0	2.0	1.4	-
	650 d)	Toxic										
CP b)	12.5	200	1	0	0	0	0	0	0.5	0.0	10.0	-

Pilot Study data:CHL Assay (Cont):

(direct method : 24 hrs)

Compound	Dose (ug/ml)	Number of Cells	Number of cells with structural aberrations						Total (ppt) (%)	Total (-ppt) (%)	Polyploid cells (%)	Final judgement
			gpp	ctb	ctc	ctd	cse	ctf				
NEG a)	0	200	0	1	1	0	0	0	1.4	1.0	0.5	-
TAL-275	56.2	200	1	2	1	0	0	0	3.5	2.0	2.0	-
	112	200	1	6	3	0	0	1	8.3	5.5	2.3	-
	225	200	3	8	13	0	0	0	10.5	9.0	2.3	-
	650 d)	Toxic										
MC b)	0.025	200	0	20	20	0	1	0	22.0	10.0	10.0	+

ctb: Chromatid break ctc: Chromatid exchange ctd: Chromosome break cse: Chromosome exchange ctf: others
 a): Solvent control

(activation method : +S9)

Compound	Dose (ug/ml)	Number of Cells	Number of cells with structural aberrations						Total (ppt) (%)	Total (-ppt) (%)	Polyploid cells (%)	Final judgement
			gpp	ctb	ctc	ctd	cse	ctf				
NEG a)	0	200	0	0	1	0	0	0	0.5	0.5	0.5	-
TAL-275	112	200	0	0	0	0	0	0	0.0	0.0	0.0	-
	225	200	0	0	1	0	0	0	0.5	0.5	0.5	-
	450 d)	200	0	0	22	0	0	0	11.5	11.5	3.5	+
	900 d)	Toxic										
CP b)	12.5	200	2	21	17	1	0	0	40.5	30.5	10.0	+

d): Not counted

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Based on the results of the pilot assay, the doses selected for the main assay were 56-450ug/ml for the direct assay and -S9 of the activation assay and, 113-900ug/ml in +S9 of the activation assay. Summary table below from the sponsor presents the results of the main assay (individual data for the chrom abs were not provided; they were however, for the pilot assay):

Compound	Concentration (µg/ml)	Exposure-Expression time (hr)	S9 Mix	Cell survival (relative)	Mitotic index (relative)	No. of aberration				% Cells with aberrations	
						Breaks		Exchanges			Total
						ct	cs	ct	cs		
DMSO	5 µl/ml	24-0	-	100	100	0	0	0	0	0	0.0
TAK-375	56.3	24-0	-	97.6	76.7	2	0	1	0	3	1.5
	113	24-0	-	90.7	63.3	5	0	2	0	7	3.5
	225	24-0	-	67.6	26.7	5	0	3	0	8	4.0
	450*	24-0	-	7.9	0.0	Toxic					
MMC	0.05	24-0	-	ND	ND	30	0	71	0	101	43.0
DMSO	5 µl/ml	48-0	-	100	100	1	0	1	0	2	1.0
TAK-375	56.3	48-0	-	89.2	73.0	3	0	1	0	4	2.0
	113	48-0	-	65.8	73.0	4	0	9	0	13	5.5
	225	48-0	-	42.2	20.6	8	0	13	0	21	9.0
	450*	48-0	-	5.0	0.0	Toxic					
MMC	0.025	48-0	-	ND	ND	24	0	66	1	91	40.0
DMSO	5 µl/ml	6-18	-	100	100	0	0	0	1	1	0.5
TAK-375	56.3	6-18	-	97.8	80.4	0	0	1	0	1	0.5
	113	6-18	-	100.2	94.1	0	0	2	0	2	1.0
	225	6-18	-	92.0	98.0	0	0	0	0	0	0.0
	450*	6-18	-	9.5	7.8	Toxic					
CP	12.5	6-18	-	ND	ND	0	0	0	0	0	0.0
DMSO	5 µl/ml	6-18	-	100	100	0	0	1	0	1	0.5
TAK-375	113	6-18	-	93.6	107.2	0	0	0	0	0	0.0
	225	6-18	-	86.8	116.9	0	0	1	0	1	0.5
	450*	6-18	+	50.9	63.9	6	0	23	0	29	11.5
	900*	6-18	+	10.4	4.8	Toxic					
CP	12.5	6-18	+	ND	ND	21	0	67	0	88	39.5

Conclusion: A tendency of TAK-375 to slightly increase the incidence of chromosomal structural aberrations was observed. At all the doses with the observed effects, the mitotic index and cell survival rate considerably decreased relative to the control value.

ND: Not determined.

*: Visible precipitation was occurred at the end of exposure period

From the above table, it can be seen that there was a dose dependent increase in number of cells with aberrations with increase in concentration in the direct assay 24 and 48hr methods and a sig increase in the 450ug/ml dose of the -S9 of the activation assay, all compared relative to the solvent control values. Note from these data that the MI in the +S9 activation assay at the 113 and 225ug/ml doses, were not decreased but increased above the solvent control value of 100%. Therefore, the results of these 2 doses are unreliable. In addition, the absence of positive finding in the -S9 activation assay is questionable since the validity of the assay is questionable because of the inappropriate use of CP as the positive control. This latter control is usually used in +S9 and therefore, the negative findings of chrom abs with this positive control are not surprising.

It is concluded that TAK375 is clastogenic in the CHL cells assay causing both structural and numerical aberrations.

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Study Title: In vivo Micronucleus in rats
Study No: M-11-18
Volume # 7; page# 147
Conducting Laboratory: 1
Date of Study Initiation/completion: Oct. 1997/Mar 1998
GLP Compliance: Yes/Japanese
QA- Reports Yes (x) No ():
Drug Lot Number: M375-002

Study Endpoint: incidence of immature erythrocytes with micronuclei.

Methodology:

- Animal species/strain: male Sprague Dawley rats/
- Dose Selection Criteria: dose range finder.
- Basis of dose selection: top dose of 2000mg/kg; doses in main study: 500, 1000, 2000mg/kg.
- Range finding studies: 4 doses were tested: 1024, 1280, 1600, 2000mg/kg. Doses were administered in 0.5%MC by oral gavage to 3 male SD rats per dose as a single daily dose for 2 days. Body wt, survival, and general observations were done 24hr after the 2nd and final dose. Clinical signs were seen in each 1 of 3 rats dosed 1600 and 2000mg/kg at 1hr after the last dose on d2. These signs included staggering gait and the high dose rat showed abnormal respiration, lateral position, lacrimation, and mydriasis at 2hr post the last dose. All signs were gone by 24hr after the last dose. Mean B.wt was decreased in the HD and did not recover by 24hr postdose. There were no deaths in any gr.
- Test Agent Stability & Solubility: solid drug substance stability: stable for ∞ at 60&40C. stability in solution: test sub stable in the 1st and 2nd solutions of the Japanese pharmacopeia for ∞ at 37C. Test sub freely soluble in DMSO and ethanol; solubility in water is small at 200ug/ml at 37C and pH 1-9.
- Vehicle control: 0.5% MC; 2 doses; p.o. to 5 m rats; blood sampled 48hr after the 1st dose.
- Positive Controls: MMC at 2mg/kg single i.v. dose; 5 rats; blood sampled 24hr postdose.
- Incubation and sampling times:
- Doses used in definitive study: based on results of the dose range finder the following doses were selected: 500, 1000, and 2000mg/kg.
- Study design: pilot dose range finder and main assay.
 - Analysis:
 - Counting method: 2000 immature erythrocytes (IE) per animal were analyzed by a fluorescent microscope }
 - Cytotoxic endpoints: incidence of immature erythrocytes with micronuclei (MNIE) was counted. The ratio of IE to total erythrocytes was done by counting 1000 erythrocytes per animal.
- Genetic toxicity endpoints results: Negative.
 - Statistical methods: standard tables of Kastenbaum & Bowman for differences in incidence of MNIE and Dunnett's test for IE ratio comparison.
- Criteria for Positive Results: positive response if is reproducible or dose dependent.

Results:

- Study Validity: valid.
- Study Outcome: Negative.

Comments:

The positive control, mitomycin C, produced the expected significant increase in MNIE relative to the negative control. Mean body wt gain in HD of 2000mg/kg in the pilot study was 8+7g compared with 23+1g in 1024mg/kg low dose and 17+4g in 1600mg/kg dose grs (i.e. a marked reduction in HD; no info provided for the negative control mean wt). In the main study, mean wt gain was reduced in all 3 drug grs, at 4+5g, 5+9g, and -3+15g in low, mid, and high dose groups relative to a mean gain of 8+3g in the negative solvent control gr, the mean gain in the positive control gr was also low at 3+2g.

Table below from sponsor presents the summary of the findings:

Title (Study No.)	Micronucleus Test in Rats Treated with TAK-375 (3645)
Test Substance	TAK-375
Vehicle	0.5% MC
Positive Control	Mitomycin C (MMC)
Test Animals	Male CD(SD)IGS rats (7 weeks old)

Compound	Dose (mg/kg/day)	No. of Doses	Route	Sampling Time after the 1st Dose (hrs)	No. of Rats	Immature Erythrocytes with Micronuclei (%)
0.5% MC	0	2	p.o.	48hr	5	0.36
TAK-375	500	2	p.o.	48hr	5	0.45 NS
	1000	2	p.o.	48hr	5	0.32 NS
	2000	2	p.o.	48hr	5	0.36 NS
MMC	2	1	i.v.	24hr	5	6.85 **
Conclusion: There were no test substance related increases in the incidence of immature erythrocytes with micronuclei. The positive control performed as expected. Therefore, the test substance is not a clastogen or a spindle poison in the systems.						

NS, not significant; **, p<0.01 (Kastenbaum and Bowman)

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Study Title: In vivo bone marrow Micronucleus in mice

Study No: M-11-20

Volume # 7; page# 177

Conducting Laboratory: 1

Date of Study Initiation/completion: Mar, 1998/Jun 1998

GLP Compliance: Yes/Japanese & FDA

QA- Reports Yes (x) No ():

Drug Lot Number: M375-002

Study Endpoint: incidence of immature erythrocytes with micronuclei.

Methodology:

- Animal species/strain: male — mice 7wks old
- Dose Selection Criteria: dose range finder.
- Basis of dose selection: top dose of 1000mg/kg; doses in main study: 250, 500, 1000mg/kg/d.
- Range finding studies: 5 mice per dose were orally administered 500, 1000, or 2000mg/kg for 2 days as a single dose per day. Doses were administered in 0.5%MC. Body wt, survival, and general observations were done 24hr after the 2nd and final dose. Clinical signs were seen in each 1 of 3 rats dosed 1600 and 2000mg/kg at 1hr after the last dose on d2. These signs included staggering gait and the high dose rat showed abnormal respiration, lateral position, lacrimation, and mydriasis at 2hr post the last dose. All signs were gone by 24hr after the last dose. Mean B.wt was decreased in the HD and did not recover by 24hr postdose.
- Test Agent Stability & Solubility: under the test article information (Appendix 1), it is stated that "stability study has not been finished" the date is Mar 16th 1998. The analysis was then completed after the experiment was completed and was shown to be stable throughout the study period. Test sub freely soluble in DMSO and ethanol; solubility in water is small at 200ug/ml at 37C and pH 1-9.
- Vehicle control: 0.5% MC; 2 doses; p.o. to 5 m mice blood sampled 48hr after the 1st dose.
- Positive Controls: MMC at 2mg/kg/d as an i.p. dose (n=5).
- Incubation and sampling times:
- Doses used in definitive study: based on results of the dose range finder the following doses were selected: 250, 500, 1000mg/kg. There were 6 animals in each the 250&500mg/kg dose grs and in negative and positive control grs, there were 7 mice in the 1000mg/kg gr as indicated above.
- Study design: pilot:dose range finder and main assay.
 - Analysis:
 - Counting method: 2000 immature erythrocytes (IE) per animal were analyzed by a fluorescent microscope 1
 - Cytotoxic endpoints: incidence of immature erythrocytes with micronuclei (MNIE) was counted. The ratio of IE to total erythrocytes was done by counting 1000 erythrocytes per animal.
- Genetic toxicity endpoints/results: **Negative**.
 - Statistical methods: standard tables of Kastenbaum & Bowman for differences in incidence of MNIE and Dunnett's test for IE ratio comparison.
- Criteria for Positive Results: positive response if is reproducible or dose dependent

Results:

- Study Validity: valid.
- Study Outcome: Negative.

Summary and Conclusion:

In the dose range finder, 1 mouse in 1000mg/kg and 3 in the 2000mg/kg grs died after the 1st dose and, 1 mouse in the 2000mg/kg died within 24hr after the 2nd dose. Based on these findings, the top dose in the main study was set at 2000mg/kg. In the main study, there were no deaths in any gr, clinical signs included decreased activity in all drug grs after the 1st and 2nd doses (it was the only sign seen after the 2nd dose in all grs). Ataxic gait occurred in the 500mg/kg gr only after the 1st dose, in the 1000mg/kg gr, loss of righting reflex and respiratory depression were seen. There were no drug related differences in mean wt in any gr. Animals were killed 24hr after the 2nd dose and femur removed from each animal and bone marrow extracted and slides prepared according to standard procedures. (table from sponsor presents the data):

Individual Data from the Micronucleus Test on TAK-375 in Mice

Compound	Dose (mg/kg/day)	No. of doses	Route	Animal No.	% Reticulocytes ¹	% Micronucleated polychromatic erythrocytes ²
0.5% MC	10 ml/kg/day	2	p.o.	1	62.7	0.10
				10	64.3	0.10
				12	59.9	0.15
				18	66.4	0.15
				20	61.5	0.05
TAK-375	250	2	p.o.	3	65.8	0.20
				4	75.6	0.05
				5	67.4	0.15
				6	68.8	0.05
				19	75.4	0.15
TAK-375	500	2	p.o.	7	58.8	0.15
				13	50.8	0.15
				21	71.5	0.10
				28	60.8	0.10
				29	65.7	0.15
TAK-375	1000	2	p.o.	8	57.2	0.10
				9	70.1	0.20
				14	70.4	0.05
				16	49.1	0.15
				22	69.0	0.15
Mitomycin C	1	1	i.p.	2	54.4	5.25
				11	34.1	4.60
				15	46.3	7.50
				17	42.0	7.15
				25	59.7	5.70

¹ based on 1000 erythrocytes, ² based on 2000 polychromatic erythrocytes

Best Possible Copy

Study Title: in vivo/in vitro UDS assay with TAK-375 in rat hepatocytes.

Study No: M-11-22

Volume # 7; page# 211

Conducting Laboratory:

Date of Study Initiation/completion: Feb, 1998/Jul, 1998

GLP Compliance: Yes/Japanese

QA- Reports Yes (x) No ():

Drug Lot Number: M375-002

Study Endpoint: induction of unscheduled DNA synthesis reflected by an increase in net nuclear grain and increased incidence of cells in repair over the negative control.

Methodology:

- Animal species/strain: rat/Sprague Dawley males; 8wk old
- Dose Selection Criteria: based on the previous in vivo rat bone marrow micronucleus test.
- Basis of dose selection: no deaths in rats after 2 day dosing upto 2000mg/kg/d dose.
- Range finding studies: not done in this study.
- Test Agent Stability & Solubility: see info in the above studies relating to the same drug lot.
- Vehicle control: 0.5% MC
- Positive Controls: in the gr that had liver perfusion 2hr after oral drug administration, 5mg/kg of dimethylnitrosamine (DMN) prepared in water or injection, was administered. In the gr that had liver perfusion 16hr after oral drug administration, had 100mg/kg of 2-acetylaminofluorene suspended in corn oil; both positive controls were given orally.
- Incubation and sampling times:
- Doses used in definitive study:
- Study design: pilot/dose range finder and main assay.
- Analysis:

- Counting method: 150 hepatocytes per animal were counted. The nuclear grains (NG) were counted under a microscope attached to a colony analyzer. The net nuclear grains (NNG) were calculated as follows:

$$\text{NNG} = \text{NG} - \text{CG (cytoplasmic grains)}$$

Also, the number of cells in repair (those showing net grains of 5 or more, were also determined.

- Cytotoxic endpoints: low count.
- Genetic toxicity endpoints/results: cells in repair due to DNA damage.
- Statistical methods: not used.
- Criteria for Positive Results: positive if the mean NNG number was ≥ 5 and the incidence of repaired cells was $\geq 20\%$ from the negative control.

Results:

No clinical signs were reported in the 1000mg/kg gr and in the 2000mg/kg the following were seen at the 2 and 16hr periods: below normal body temperature (no value was provided), decreased motor activity, nasal discharge and lateral position, at 16hr period abnormal gait was also reported. Mean wt and wt gain were higher in drug groups relative to the negative control at the 2hr postdose period but there was a mean gain loss (dose-dependent) at the 16hr period at time of sacrifice (293 $\pm$ 7g control, 286 $\pm$ 12g in 1000mg/kg, and 276 $\pm$ 13g in 2000mg/kg gr). Table below from sponsor summarizes the findings of induction of UDS:

Compound	Dose (mg/kg)	Volume (mL/kg)	No. of doses	Route	No. of animals evaluated	Duration of treatment (hr)	Net nuclear grains (NNG) Mean ± S.D.	Cells in repair (%) Mean ± S.D.
0.5% MC	0	10	1	p.o.	3	2	-1.3±0.1	0.0±0.0
						16	-0.9±0.5	1.1±0.4
TAK-375	1000	10	1	p.o.	3	2	-1.2±1.0	2.0±0.7
						16	-0.3±0.5	2.7±0.7
	2000	10	1	p.o.	3	2	-1.1±0.6	3.8±0.4
						16	-0.3±0.4	4.0±0.0
DMN	5	10	1	p.o.	3	2	30.6±1.0	100.0±0.0
2AAF	100	10	1	p.o.	3	16	29.0±1.6	100.0±0.0

There seemed to be no difference in NNG of drug treated and negative control gr however, the number of cells in repair increased dose-dependently in drug groups relative to the control. This was also evident from the individual data at both time points. The positive control had a marked increase in NNG and 100% of cells were in repair. Therefore, it can be concluded that TAK375 was mutagenic in the UDS assay though the response was weak (tables from sponsor).

Individual data of UDS incidence in hepatocytes from rat treated *in vivo* with TAK-375 (2hr post-treatment)

Compound	Dose (mg/kg)	Animal ID No.	Observed Cells	Nuclear grains Mean ± S.D.	Cytoplasmic grains Mean ± S.D.	Net nuclear grains Mean ± S.D.	Cells in repair (%) a)
0.5% MC b)	0	1001	150	1.25 ± 2.58	0.64 ± 3.03	-1.29 ± 2.70	0.0
		1003	150	0.61 ± 2.29	7.87 ± 1.99	-1.28 ± 2.89	0.0
		1004	150	0.89 ± 2.35	0.17 ± 3.00	-1.48 ± 2.90	0.0
TAK-375	1000	1101	150	0.11 ± 2.95	6.27 ± 1.36	-0.18 ± 3.18	1.3
		1103	150	1.47 ± 2.92	0.55 ± 4.06	-2.87 ± 3.95	2.7
		1104	150	1.25 ± 2.69	0.70 ± 3.28	-1.45 ± 3.23	2.0
	2000	1201	150	1.54 ± 2.68	0.73 ± 2.85	-1.25 ± 3.34	4.0
		1202	150	7.23 ± 3.60	0.33 ± 4.62	-1.80 ± 4.03	4.0
		1203	150	1.32 ± 2.58	7.71 ± 2.85	-0.45 ± 2.87	3.3
DMN c)	5	1301	150	36.27 ± 1.62	6.17 ± 2.77	30.10 ± 1.50	100.0
		1302	150	35.49 ± 1.74	5.57 ± 3.40	29.91 ± 1.21	100.0
		1304	150	39.45 ± 6.87	7.67 ± 3.03	31.77 ± 6.87	100.0

a) : UDS positive cells ; Percent of cells having 5 or greater net nuclear grains
 b) : Vehicle control
 c) : Positive control (DMN : Dimethylnitrosamine)

Individual data of UDS incidence in hepatocytes from rat treated *in vivo* with TAK-375 (16hr post-treatment)

Compound	Dose (mg/kg)	Animal ID No.	Observed Cells	Nuclear grains Mean ± S.D.	Cytoplasmic grains Mean ± S.D.	Net nuclear grains Mean ± S.D.	Cells in repair (%) a)
0.5% MC b)	0	2001	150	5.73 ± 2.55	0.89 ± 3.28	-1.10 ± 2.18	0.7
		2002	150	5.48 ± 2.11	5.89 ± 2.84	-0.39 ± 2.84	1.3
		2004	150	5.42 ± 2.20	0.05 ± 3.19	-1.23 ± 3.23	1.3
TAK-375	1000	2101	150	0.47 ± 3.16	0.87 ± 3.70	-0.38 ± 3.01	3.3
		2102	150	0.90 ± 3.95	10.05 ± 4.37	-0.75 ± 3.58	2.7
		2104	150	0.30 ± 2.51	0.15 ± 2.97	-0.15 ± 2.63	2.0
	2000	2201	150	0.23 ± 3.54	0.61 ± 4.00	-0.37 ± 3.47	4.0
		2203	150	0.85 ± 2.58	7.51 ± 3.67	-0.68 ± 3.65	4.0
		2204	150	0.11 ± 2.48	6.01 ± 2.92	0.10 ± 2.90	4.0
2AAF c)	100	2301	150	39.66 ± 1.58	0.70 ± 3.33	38.97 ± 0.74	100.0
		2302	150	37.02 ± 6.53	0.75 ± 3.62	36.27 ± 6.61	100.0
		2303	150	35.91 ± 1.44	0.81 ± 3.43	35.10 ± 1.89	100.0

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BMS incidence in hepatocytes from rat treated *in vivo* with TAK-375
 (2hr post-treatment)

Compound	Dose (mg/kg)	Animal ID-No.	Chamber	Observed Cells	Nuclear grains Mean	Cytoplasmic grains Mean	Net nuclear grains Mean	No. of cells in repair a)
0.5% VC b)	0	1001	1	50	6.72	7.40	-0.68	0
			2	50	6.64	7.64	-1.00	0
			3	50	7.39	8.88	-1.50	0
		1003	1	50	6.90	7.78	-1.78	0
			2	50	6.97	7.88	-0.98	0
			3	50	6.90	7.90	-1.00	0
		1004	1	50	6.22	7.68	-1.46	0
			2	50	7.48	8.30	-1.02	0
			3	50	6.36	7.52	-1.16	0
TAK-375	1000	1101	1	50	5.56	6.10	-0.54	1
			2	50	5.36	6.48	-1.10	1
			3	50	6.00	6.24	0.24	0
		1103	1	50	7.64	8.60	-1.02	0
			2	50	7.54	10.00	-2.54	3
			3	50	7.24	9.00	-1.76	1
		1104	1	50	7.16	8.82	-1.66	1
			2	50	7.32	8.40	-1.08	2
			3	50	7.28	8.88	-1.60	0
	2000	1201	1	50	7.78	8.38	-0.60	4
			2	50	8.30	8.52	-0.22	3
			3	50	8.02	8.18	-0.16	1
		1202	1	50	7.08	8.82	-1.74	2
			2	50	7.38	8.38	-1.00	1
			3	50	7.60	8.16	-0.56	3
		1203	1	50	8.74	7.32	1.42	2
			2	50	7.98	7.80	0.18	3
			3	50	7.24	8.28	-1.04	0

a) : BMS positive cells : Percent of cells having 5 or greater net nuclear grains
 b) : Vehicle control

BMS incidence in hepatocytes from rat treated *in vivo* with TAK-375
 (18hr post-treatment)

Compound	Dose (mg/kg)	Animal ID-No.	Chamber	Observed Cells	Nuclear grains Mean	Cytoplasmic grains Mean	Net nuclear grains Mean	No. of cells in repair a)
0.5% VC b)	0	2001	1	50	5.30	6.36	-1.06	1
			2	50	6.24	7.78	-1.54	0
			3	50	5.84	6.54	-0.70	0
		2002	1	50	5.50	6.36	-0.86	1
			2	50	5.70	5.46	0.24	7
			3	50	5.80	5.84	-0.04	0
		2004	1	50	5.06	7.82	-2.76	0
			2	50	6.06	6.80	0.74	2
			3	50	5.72	7.14	-1.42	0
TAK-375	1000	2101	1	50	6.02	8.56	-2.54	1
			2	50	7.94	8.42	-0.48	2
			3	50	8.46	8.62	-0.16	3
		2102	1	50	10.38	10.68	-0.30	1
			2	50	10.10	10.74	-0.64	7
			3	50	9.22	10.74	-1.52	0
		2104	1	50	9.40	8.90	0.50	2
			2	50	8.16	8.38	-0.22	1
			3	50	7.34	7.16	0.18	0
	2000	2201	1	50	8.38	8.38	0.00	2
			2	50	8.62	8.38	0.24	3
			3	50	7.28	8.12	-0.84	1
		2203	1	50	8.98	6.94	2.04	3
			2	50	7.38	8.50	-1.12	2
			3	50	8.30	7.18	1.12	1
		2204	1	50	6.32	4.44	1.88	3
			2	50	6.52	5.48	1.04	2
			3	50	5.48	5.42	0.06	2

a) : BMS positive cells : Percent of cells having 5 or greater net nuclear grains
 b) : Vehicle control

The following studies are taken from review of serial submission #013 by Aisar Atrakchi, Ph.D. and involve the determination of TAK-375 metabolites in the S9 reaction mix solutions used in *in vitro* genetic toxicology assays.

8. Concentrations of metabolites M-I, M-II, M-III, and M-IV of TAK 375 in the reaction mixture for the bacterial reversion assay after incubating TAK 375 with S9/M-11-189/study# 3110/ge

Conducting lab: Takeda, Japan

GLP: No

QA report: Yes

Initiation/Termination dates: Sep 1999

Drug lot#/purity: M-375-013 L }
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 }
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Doses & vehicle: 156, 1250, and 5000ug/ml; the control was DMSO.

This study was conducted to determine the concentrations of TAK-375 metabolites in the reaction mixture of the in vitro bacterial reverse gene mutation assay conducted previously. [the reviewer is unclear as to the specific purpose of measuring the concentrations of these metabolites in this assay specially since it is known that these metabolites are formed by liver S9 in vivo. Therefore, it is expected/assumed that they will be present in an in vitro system that uses S9 mix].

Methods and Results:

The same culture medium, drug concentrations, and other experimental conditions were used in this assay as the previously conducted one. DMSO was the vehicle control. Aliquot of TAK 375 or DMSO plus S9, plus culture medium were placed in a test tube, mixed and then incubated for 20min with gentle shaking. There were 30 tubes per concentration. Following incubation, tubes were processed and concentrations of M-I, M-II, M-III, and M-IV were determined using HPLC with detection limit of $\sim$ g/ml.

Table below from the sponsor summarizes the results:

Concentrations of Metabolites M-I, M-II, M-III and M-IV of TAK-375 in the Reaction Mixture for the Bacterial Reversion Assay after Incubating TAK-375 with S9 mix (3110/ge)

Concentration of TAK-375 in		Concentration (ng/mL, n=3)			
dosing solution (mg/mL)	reaction mixture (mg/mL)	M-I	M-II	M-III	M-IV
0	0	< 5	< 5	< 5	< 5
156	0.223	165	5629	257	< 5
12.5	1.79	81	8772	650	< 5
50	7.14 [†]	188	12184	6854	< 5

[†] Precipitation was seen.

7. Concentrations of metabolites M-I, M-II, M-III, and M-IV of TAK 375 in the reaction mixture for the in vitro MLP assay after incubating TAK 375 with S9/M-11-186/study# 3112/ge

Conducting lab: Takeda, Japan

GLP: No

QA report: Yes

Initiation/Termination dates: Sep 1999

Drug lot#/purity: M-375-013 []

Doses & vehicle: 100, 200, and 400ug/ml; the control was DMSO.

This study was conducted to determine the concentrations of TAK-375 metabolites in the reaction mixture of the in vitro mouse lymphoma assay conducted previously. [the reviewer is unclear as to the specific purpose of measuring the concentrations of these metabolites in this assay specially since it is known that these metabolites are formed by liver S9 in vivo. Therefore, it is expected/assumed that they will be present in an in vitro system that uses S9 mix].

Methods and Results:

The same culture medium, drug concentrations, and other experimental conditions were used in this assay as the previously conducted one. DMSO was the vehicle control. Aliquot of F0P and F10P culture medium plus S9 were placed in a centrifuge tube, the drug at each concentration or DMSO, were added to this mixture and mixed briefly. This mixture was incubated for 4hrs and mixed gently. Any precipitation was checked by the naked eye when the drug was added to the mixture and after the 4hr incubation period. Concentrations of M-I, M-II, M-III, and M-IV were then determined using HPLC with detection limit of — g/ml.

Table below from the sponsor summarizes the results:

Concentrations of Metabolites M-I, M-II, M-III and M-IV of TAK-375 in the Reaction Mixture for the Mouse Lymphoma Assay after Incubating TAK-375 with S9 mix (3112/ge)

Concentration of TAK-375 in		Concentration (ng/mL, n=3)			
dosing solution (mg/mL)	reaction mixture (ug/mL)	M-I	M-II	M-III	M-IV
0	0	0	0	0	0
10	100	3702	1953	6081	2011
20	200	5336	11102	17086	1956
40	400	5168	25626	33861	1031

Values less than the quantitation limit (— ng/mL) were presumed to be 0.

6. Concentrations of metabolites M-I, M-II, M-III, and M-IV of TAK 375 in the reaction mixture for the in vitro cytogenetic assay after incubating TAK 375 with S9/M-11-185 study# 3108/ge

Conducting lab: Takeda, Japan

GLP: No

QA report: Yes

Initiation/Termination dates: Sep 1999

Drug lot#/purity: M-375-013 []

Doses & vehicle: 11.3, 22.5, and 45mg/ml; the control was DMSO.

This study was conducted to determine the concentrations of TAK-375 metabolites in the reaction mixture of the in vitro cytogenetic Chinese hamster lung cells assay conducted previously. [the reviewer is unclear as to the specific purpose of measuring the concentrations of these metabolites in this assay specially since it is known that these metabolites are formed by liver S9 in vivo. Therefore, it is expected/assumed that they will be present in an in vitro system that uses S9 mix].

Methods and Results:

The same culture medium, drug concentrations, and other experimental conditions were used in this assay as the previously conducted one. DMSO was the vehicle control. The TAK 375 concentrations were the same as those used previously at the 6hr treatment in +S9. The drug at each concentration (50ul), or DMSO, was mixed with 5ml culture medium and S9 at 16.7% and placed into plastic petri dishes (triplicate cultures), and incubated for 6hrs under 5% CO2 gas. Any precipitation was checked by the naked eye when the drug was added to the mixture and after the 6hr incubation period. Concentrations of M-I, M-II, M-III, and M-IV were then determined using HPLC with detection limit of 0 ng/ml.

Table below from the sponsor summarizes the results:

Concentration of TAK-375 in		Concentration (ng/mL, n=3)			
dosing solution (mg/mL)	reaction mixture (mg/mL)	M-I	M-II	M-III	M-IV
0	0	0	0	0	0
11.3	0.113	7644	1141	5935	883
22.5	0.225	12423	3699	18228	677
45.0	0.450 [†]	9971	7249	26172	262

Values less than the quantitation limit (0 ng/mL) were presumed to be 0

[†] Precipitation was observed.

2.6.6.5 Carcinogenicity

Study title: Twenty-four month oral gavage carcinogenicity study of TAK-375 in mice

Key study findings:

- B6C3F₁ mice (n = 55 mice/sex/group) were administered vehicle (0.5 w/v% methylcellulose) or TAK-375 at doses of 30, 100, 300 or 1000 mg/kg/day by oral gavage for up to 104 weeks. Separate animals were used for toxicokinetic analysis (n = 60 mice/sex/group) and received 30, 100, 300 or 1000 mg/kg/day for 52 weeks and plasma levels of TAK-375 and 4 metabolites, including the active metabolite M-II were analyzed from Day 1, Week 13 and Week 52.
- TAK-375 administration at a dose level of 1000 mg/kg/day produced excessive mortality with survival rate in this group beginning to decline markedly after week 70 in both sexes and requiring the early termination of this dose group after Week 94 of administration. Clinical signs observed were typically decreased spontaneous movement, prone or lateral position, slow and shallow breathing, and abdominal

distension. Presumptive cause of death in all early decedents was largely attributed to liver tumor.

- Body weight was significantly decreased in males and females given 1000 mg/kg/day compared with controls at Week 94 (-18% and -15%, respectively). Body weight gain over 104 weeks was decreased in males at 300 mg/kg/day (-12%) but females at this dose level were not significantly different from controls. High dose groups were not analyzed due to early termination of these groups. Of note, in the face of this significant reduction in body weight food consumption markedly increased in 1000 mg/kg/day group animals starting approximately after 65 weeks and reaching maximum around Week 81.
- Hematologic alterations were associated with dosing ≥ 300 mg/kg/day in male and female mice and included increased mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) in males and increased platelets in males and females. The 1000 mg/kg/day dose level was associated with increased RBCs (female), hemoglobin, hematocrit, mean cell volume (females), MCH, MCHC, reticulocytes (males) and platelets.
- TAK-375 administration was associated with the development of tumors of the liver in males (for dose levels 0, 30, 100, 300 and 1000 mg/kg/day incidence was - adenoma: 20/55, 24/55, 32/55, 50/55* and 54/55*; carcinoma: 9/55, 6/55, 18/55, 26/55* and 50/55*; hepatoblastoma: 0/55, 0/55, 1/55, 3/55* and 10/55*) and females (adenoma: 10/55, 14/55, 14/55, 52/55* and 55/55*; carcinoma: 4/55, 3/55, 3/55, 11/55 and 51/55*). Combining the incidence of hepatocellular adenoma and carcinoma in males reduced the dose level at which significant difference from control was found to 100 mg/kg/day, however, while females remained at 300 mg/kg/day. Harderian gland adenoma appeared elevated in treated groups relative to controls but trend analysis did not reach statistical significance.
- Non-neoplastic findings in the liver which were elevated in TAK-375-treated groups relative to control animals and showed evidence of dose-dependence included hepatocellular hypertrophy, altered cell focus, single cell necrosis (males), focal necrosis (females), and centrilobular necrosis (males), and pigmentation of sinusoidal lining. Findings in other tissues included adrenal cortical cell hypertrophy, lymphocytic cell infiltration of the epididymis and atrophy of the seminal vesicle, glomerulosclerosis (males), ovarian, uterine and vaginal atrophy, extramedullary hematopoiesis in the spleen, squamous cell hyperplasia of the forestomach with inflammatory cell infiltration and hypertrophy of thyroid follicular cells.
- When hepatic adenoma and carcinoma were analyzed separately, the liver NOAEL dose for adenoma or carcinoma (100 mg/kg/day) was associated with a plasma concentration of TAK-375 which provides a margin of safety above the expected human therapeutic exposure of 1,079- and 827-fold based on male and female mouse exposure. Similarly, the liver NOAEL dose in male and female mice was associated with plasma concentrations of the active metabolite M-II which provide a safety margin for human exposure at the expected therapeutic concentrations of approximately 30- and 12-fold from male and female mice, respectively. If the incidences of hepatic tumors are analyzed together, the lowered NOAEL dose in the male provides for a safety margin of 103-fold for TAK-375 and 3-fold for the M-II metabolite over the expected human exposure.

- Extrapolation of these findings to humans is uncertain as xenobiotics which produce hepatic enzyme induction are known to produce similar hepatic tumors in rodents while these drugs are not associated with tumor development in the human. Regarding tumors of the Harderian gland, a comparable organ does not exist in humans rendering this finding of questionable predictive value. However, in genetic toxicology assays TAK-375 has been demonstrated to be clastogenic in an *in vitro* chromosomal aberration assay and positive, although weakly so, in an Unscheduled DNA Synthesis assay which could indicate a genotoxic mechanism for tumor development.

Study no.: M-11-00560.002A (includes amendments)

Volume #, and page #: N/A

Conducting laboratory and location: []

Date of study initiation: October 4, 1999

GLP compliance: Compliant with Japanese GLP guidelines

QA report: yes (X) no ()

Drug, lot #, and % purity: TAK-375, Lot M375-017, [] 1% purity; certificate of analysis included. Vehicle is methylcellulose [] SM-100), lot #806611, 911648, 002658 and 104658.

CAC concurrence: No. See Appendix 1.

Methods

Doses: Vehicle (0.5 w/v% methylcellulose solution), 30, 100, 300 or 1000 mg/kg/day. See table below for basic design of study.

Test group	Dose Level (mg/kg/day)	Concentration of test suspension (mg/ml.)	Dose volume (ml/kg)	Sex	Main Group		Satellite Group	
					No. of animals	Animal number	No. of animals	Animal number
Control	0	0	10	Male	55	1001-1055	—	—
				Female	55	1101-1155	—	—
Low	30	3	10	Male	55	2001-2055	67	2201-2267
				Female	55	2101-2155	67	2301-2367
Middle	100	10	10	Male	55	3001-3055	67	3201-3267
				Female	55	3101-3155	67	3301-3367
High	300	30	10	Male	55	4001-4055	67	4201-4267
				Female	55	4101-4155	67	4301-4367
Highest	1000	100	10	Male	55	5001-5055	67	5201-5267
				Female	55	5101-5155	67	5301-5367

Basis of dose selection: Doses were selected based an estimation of the MTD from a 4-week toxicity study and a 13-week toxicokinetic dose range-finding study. The 4-week study utilized oral dosing of 0, 50, 150 500 or 1500 mg/kg/day TAK-375. Decreased spontaneous movement was observed at 500 mg/kg/day while at 1500 mg/kg/day 3/5 males within this group died in the post-dosing period indicating excessive toxicity. A subsequent 13-week dose range-finding study for the 2-year bioassay identified evidence of hepatic enzyme induction in male and female mice with

dosing ≥ 100 mg/kg/day, decreased spontaneous movement and histopathologic evidence of hepatic enzyme induction (centrilobular hypertrophy) along with in vitro demonstration of increased enzymatic activity at dose levels ≥ 300 mg/kg/day, while the high dose of 1000 mg/kg/day was associated with the death of a single male mouse in the immediate post-dosing period on Day 2 of the study. The Sponsor noted that evidence of reduced plasma exposure to TAK-375 was observed over the course of the study and believed that during the course of a 2-year study, an initial loss of mice may be expected with the proposed high dose of 1000 mg/kg/day but would likely not be maintained throughout the study. Dosing of other groups was determined by a common factor of 3.3.

Species/strain: — B6C3F₁ SPF mice ♂

Number/sex/group (main study): 55 mice/sex/group

Route, formulation, volume: oral gavage, 10 mL/kg

Frequency of dosing: Daily

Satellite groups used for toxicokinetics or special groups: 67 mice/sex/group used for toxicokinetic analysis

Age: 6 weeks at initiation of study; weights 20.7 – 26.6 g (♂), 15.7 – 21.7 g (♀)

Animal housing: Males and females in separate rooms, Temp. 19 – 27°C, relative humidity 39 – 84%, air ventilation 10 – 15 times per hour and 12-hour light cycle (7 a.m. to 7 p.m.) Animals were housed individually in stainless steel wire mesh cages.

Restriction paradigm for dietary restriction studies: N/A; food and water available *ad libitum*.

Drug stability/homogeneity: Dosing suspension maximally made 8 days in advance and 7 days suspension made at a time. Stability study demonstrated that suspensions between 0.1 and 100 mg/mL maintained concentrations to within — of the initial concentration by Day 8 after sitting — at room temperature following 7 days of refrigeration. Concentration and uniformity of dosing suspensions were determined at the start of the study and then — until the conclusion of the study. All dosing suspensions were within 10% of nominal concentration, range []%. Uniformity ranged from [] Studies were supplied as attached data reports.

Dual controls employed: No.

Interim sacrifices: Not conducted, though high dose group was sacrificed during week 95.

Statistical methods: Taken verbatim from Sponsor's submission:

Survival: Survival curves were analyzed using the Kaplan-Meier's method while the trend of survival rate to dose level was analyzed by Tarone-type method. The difference between control and each dose group was compared using a log-rank test. The lowest level of significance was 5% in two-tailed level.

Body weight, Food Consumption and Hematologic Data: A Bartlett's test for homogeneity of variance was first used. When this assumption was demonstrated accurate a Williams' test, which assumes a dose-related trend was performed to determine the non-toxic dose level. If there were no significant differences a Dunnett's test was used to compare the mean in the control with that in each other dose group. When variances were no homogeneous the Shirley-Williams test, which assumes a dose-related trend, was performed to determine the non-toxic level and the Steel test was performed to compare the mean rank in the control group with that in each dose group. Bartlett's test was conducted at the two-tailed significance level of 0.05, the Williams' and Shirley-Williams tests were conducted at the two-tailed significance level of 0.05 and the other tests were conducted at the two-tailed significance levels of 0.05 and 0.01.

Tumor Incidence: Tumors that occurred with a frequency of more than 5% in either the control or any dose group were assessed by Peto's method to assess dose-dependency for all groups and to compare the incidence between the control group and each dose group (level of significance: 5% one-tailed level). In addition, Fisher's exact test was applied to assess the difference of tumor incidence between the control group and each dose group (level of significance: 5% one-tailed level).

Note: Non-neoplastic histopathologic findings were not subject to statistical analysis.

Deviations from original study protocol:

1. High dose animals were sacrificed during Week 95 of the study.
2. Occasional animal room deviations from prespecified humidity boundaries and temperature. No deviations were long in effect (>3 hr) or great in severity.

Observation times

Mortality: All animals were observed for morbidity and mortality 3 times per day except on Saturdays and holidays in which they were observed twice per day.

Clinical signs: As above, animals were observed three times daily, once prior to dosing, immediately after dosing and 1 hr after dosing. On Saturdays and holidays animals were observed twice per day, prior to dosing and immediately after dosing. Palpation was done once a week to detect any superficial tumors.

Body weights: Twice during week 1 (Day 1 and Day 7) then once weekly thereafter at 7 day intervals. Measurement taken between 8 a.m. and 11 a.m., prior to dosing.

Food consumption: Twice during week 1 (Day 1 and 7) then once weekly thereafter at 7 day intervals with daily average determined from a 7 day food consumption total.

Clinical pathology: Upon sacrifice, blood was collected from the abdominal aorta (non-fasting) and the following parameters were determined: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte ratio, platelet count, white blood cell count and differential leukocyte count.

Gross pathology: All organs/tissues in the cephalic, thoracic and abdominal cavity were examined macroscopically for gross lesions.

Histopathology: Peer review: yes (X), no ()

1). No signed confirmation of conclusions. \

The following organs/tissues were assessed microscopically: cerebral cortex, cerebellum, spinal cord (cervical, thoracic, lumbar), sciatic nerve**, eyes*, Harderian glands*, pituitary, thyroids (including parathyroids)*, adrenals*, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, thoracic aorta, trachea, lungs (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular glands*, sublingual glands*, liver, gallbladder, pancreas, kidneys*, urinary bladder, testes*, epididymides*, prostate, seminal vesicles*, ovaries*, uterus (both horns), vagina, mammary gland (females)*, sternum (including bone marrow), femur (including bone marrow)**, femoral muscle (skeletal muscle)**, skin (inguinal)**, gross lesions, extraorbital lacrimal glands on both sides, Zymbal's glands (both sides), oviducts (both sides), nasal cavity and larynx. Single asterisk indicates that the pair of organs were examined while a double asterisk indicates that the pair of organs/tissues were removed but one side was examined while the other was preserved for examination if necessary.

Toxicokinetics: Blood samples were collected for analysis from satellite toxicokinetic mice on Day 1, Week 13 (86th dosing day for males, 85th dosing day for females) and Week 52 (359th dosing for males and 358th dosing for females). Samples were collected from the inferior vena cava after sacrifice at 6 (Day 1) or 7 (Week 13 and 52) time points (prior to dosing (not on Day 1), 0.25, 0.5, 1, 3, 6, and 24 hr after dosing). 3 animals/sex/group were used for each time-point. Samples were sent to Takeda Analytical Research Laboratories, Ltd. for determination of plasma TAK-375 levels as well as the metabolites M-I, M-II, M-III and M-IV. C_{max} was determined as well as T_{max} and the AUC_{0-24} was calculated using the trapezoidal method.

Results

Mortality:

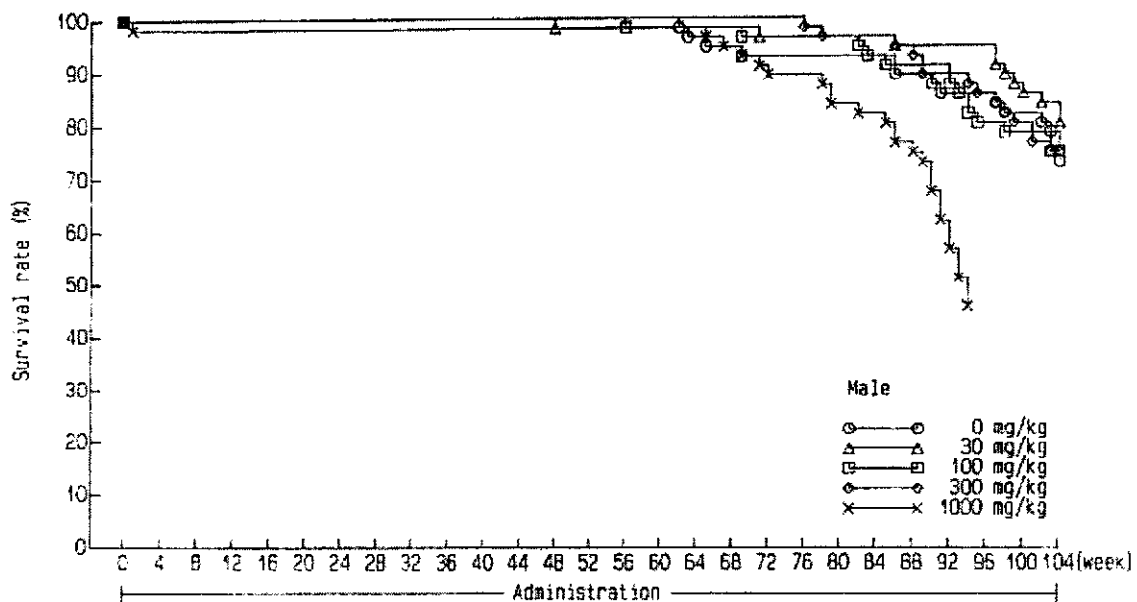


Fig 1 A 24-month oral carcinogenicity study of TAK-375 in mice

— Survival rate —

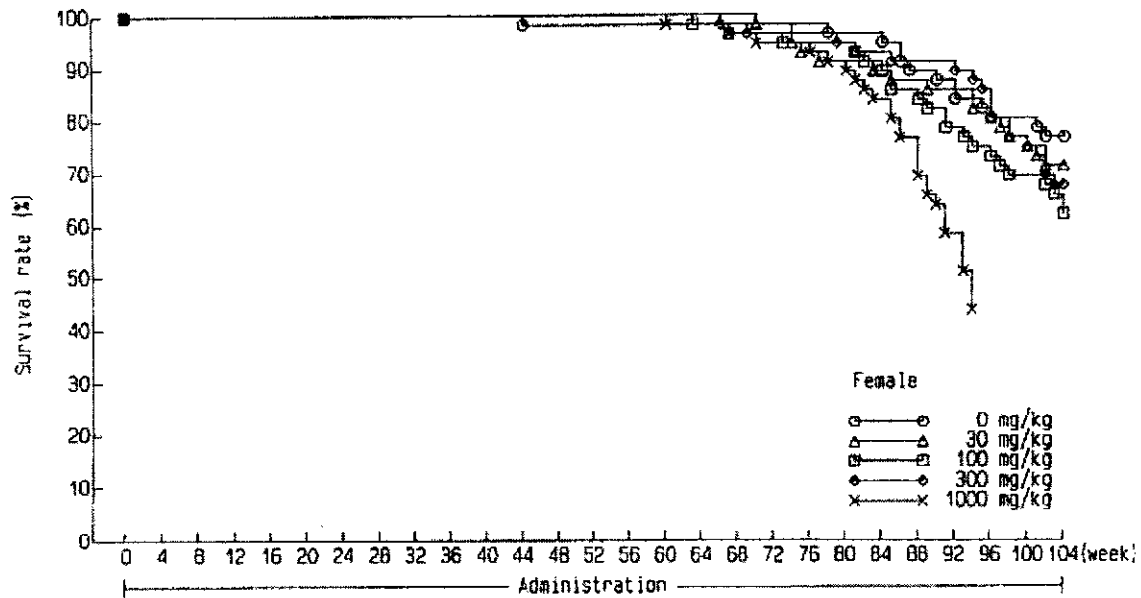


Fig.2 A 24-month oral carcinogenicity study of TAK-375 in mice

— Survival rate —

Text Table I. Summary of survival rate

Sex	Male					Female				
	0	30	100	300	1000 ^{a)}	0	30	100	300	1000 ^{a)}
Dose (mg/kg/day)	0	30	100	300	1000 ^{a)}	0	30	100	300	1000 ^{a)}
No. of animals used	55	55	55	55	55	55	55	55	55	55
Week of administration										
26	0 ^{b)}	0	0	0	1	0	0	0	0	0
52	0	1	0	0	1	1	0	0	0	0
78	4	2	2	2	7	2	5	3	2	5
104	15	11	14	14	30 ^{c)}	13	16	21	18	31 ^{c)}
Survival rate (%)	72.7	80.0	74.5	74.5	45.5*	76.4	70.9	61.8	67.3	43.6*

a): Necropsied after week 94 of administration (in week 95 of administration).

b): Cumulative number of deaths

c): Including 2 males (Nos. 5017 and 5033) and one female (No. 5120) that died or were sacrificed moribund during the necropsy period (week 95 of administration).

*: p<0.05 (significantly different from the control group)

Incidences of premature mortality were generally infrequent and a significant effect of TAK-375 on survival was only noted in the 1000 mg/kg/day group males and females though a non-significant trend towards dose-related mortality may be observed in females towards the end of the study at lower dose levels. Animals in the 1000 mg/kg/day groups were, however, terminated during week 95 to preserve a sufficient number of animals in this group for post-mortem analysis.

Text Table 2. Presumptive cause of demise

Sex	Male					Female				
	0	30	100	300	1000	0	30	100	300	1000
Dose (mg/kg/day)										
No. of animals used	55	55	55	55	55	55	55	55	55	55
No. of deaths	15	11	14	14	30	13	16	21	18	31
Tumor										
Blood vessel tumor	2	4	0	0	0	0	2	0	1	0
Bone tumor	0	0	0	0	0	0	0	0	1	0
Harderian gland tumor	0	0	0	0	0	0	1	0	0	0
Histiocytic tumor	4	0	2	0	1	4	5	2	4	0
Liver tumor	2	2	8	10	26	1	2	2	2	30
Lung tumor	2	1	0	0	0	0	0	2	0	0
Malignant lymphoma	2	1	2	1	0	6	4	10	9	0
Pituitary tumor	0	0	0	0	0	1	0	1	0	0
Skin/subcutaneous tumor	0	0	0	0	0	0	0	1	1	0
Total deaths of tumor	12	8	12	11	27	12	14	18	18	30
Non-tumor										
Amyloidosis	0	0	0	0	0	0	1	1	0	0
Diaphragmatic hernia	1	0	0	0	0	0	0	0	0	0
Intestinal lesion	1	0	0	0	0	1	0	0	0	0
Urogenital tract lesion	1	2	2	3	1	0	1	2	0	0
Total deaths of non-tumor	3	2	2	3	1	1	2	3	0	0
Test substance toxicity	0	0	0	0	1	0	0	0	0	0
Unclear	0	1	0	0	1	0	0	0	0	1

Post-mortem examination of animals found dead or sacrificed due to poor condition revealed several differences in the assumed cause of death. Primarily, however, death was believed due to tumor formation. Less than 20% of animals were thought to have died or been sacrificed due to non-tumor-related reasons in any dose group. At the TAK-375 1000 mg/kg/day level 96% of males and 100% of female premature deaths were thought to be related to tumors. The type of tumors involved in early termination or premature death were altered with increasing TAK-375 dose such that hepatic tumors accounted for 87% (26/30) of deaths in the males and 97% (30/31) of deaths in the females at the 1000 mg/kg/day level. At lower TAK-375 levels, females were observed to have a higher incidence of malignant lymphoma and histiocytic tumors when compared with control though this may be evidence of background levels and the earlier deaths of high dose females may explain the lack of this finding in these animals. Males in control group and lower dose levels had a higher incidence of malignant lymphoma, blood vessel tumors, lung tumors and histiocytic tumors contributing to premature death compared with the 1000 mg/kg/day males.

Clinical signs:

Clinical observation revealed TAK-375-related effects only at the 1000 mg/kg/day dose level in both male and female mice. These effects are delineated in the Sponsor's table below and involved increased observational incidence of reduced spontaneous activity, prone/lateral position, shallow/slower breathing and, in females, distension of the abdomen. Observation of apparent hypothermia were more common in male mice given TAK-375 than lower dose levels or controls while in female mice this observation was less pronounced compared with lower TAK-375 dose levels, though treated groups had a higher incidence than controls. The incidence of decreased spontaneous activity, hypopnea and prone/lateral position was primarily

a response observed in the first week of dosing and in both males and females disappeared during week 2 with occasional resurfacing in males after week 15 and in females after week 35. The Sponsor suggested that the abdominal distension observed in the 1000 mg/kg/day dose level in both males and females was a secondary consequence associated with the development of liver tumors in these animals. No effects were noted with dosing levels ≤ 300 mg/kg/day in either male or female mice.

Text Table 3. Incidence of main clinical signs

Sex	Males					Females				
	0	30	100	300	1000 ^{a)}	0	30	100	300	1000 ^{a)}
Dose (mg/kg/day)										
No. of animals used	55	55	55	55	55	55	55	55	55	55
Decrease, spontaneous movement	6	4	4	5	47	5	5	5	6	26
Prone/lateral position	2	0	3	1	35	1	4	2	2	26
Oligopnea	7	3	7	6	43	7	10	8	11	36
Hypothermia	6	4	6	5	13	7	10	6	10	12
Opacity, eyeball	1	3	2	6	3	0	2	2	1	0
Distention, abdomen	6	3	9	10	36	5	8	10	13	33
Excoriation	4	1	8	3	2	1	2	1	1	2

a): Necropsied after week 94 of administration (in week 95 of administration).

Detailed clinical examination for superficial tumors revealed no evidence of a dose-related increase in detectable masses as can be seen in Sponsor's table reproduced below.

Text Table 4. Incidence of palpable mass-bearers

Sex	Males					Females				
	0	30	100	300	1000 ^{a)}	0	30	100	300	1000 ^{a)}
Dose (mg/kg/day)										
No. of animals used	55	55	55	55	55	55	55	55	55	55
No. of palpable mass-bearers	4	6	3	3	1	0	4	7	4	1

a): Necropsied after week 94 of administration (in week 95 of administration).

Body weights:

Body weight was unaffected by TAK-375 administration with dosing up to 100 mg/kg/day in males and 300 mg/kg/day in females. Male mice administered TAK-375 at 300 mg/kg/day had a statistically significant reduction in body weight gain during the course of the study when compared with controls (-12%) while males receiving TAK-375 at 1000 mg/kg/day exhibited a significant reduction in body weight (-18%) compared with controls at week 94, corresponding to the early termination of this dose level (see Sponsor's Table 5, below).

Text Table 5. Summary of body weight

Sex	Males				Females			
	30	100	300	1000 ^{a)}	30	100	300	1000 ^{b)}
Dose (mg/kg/day)	30	100	300	1000 ^{a)}	30	100	300	1000 ^{b)}
No. of animals used	55	55	55	55	55	55	55	55
Mean body weight at the end of administration	N	N	N	-18% ^{*b)}	N	N	N	-15% ^{*b)}
Body weight gain (0 - 104 w)	N	N	-12% [*]	NA	N	N	N	NA

Values in the table indicate change of percentage against the mean control value.

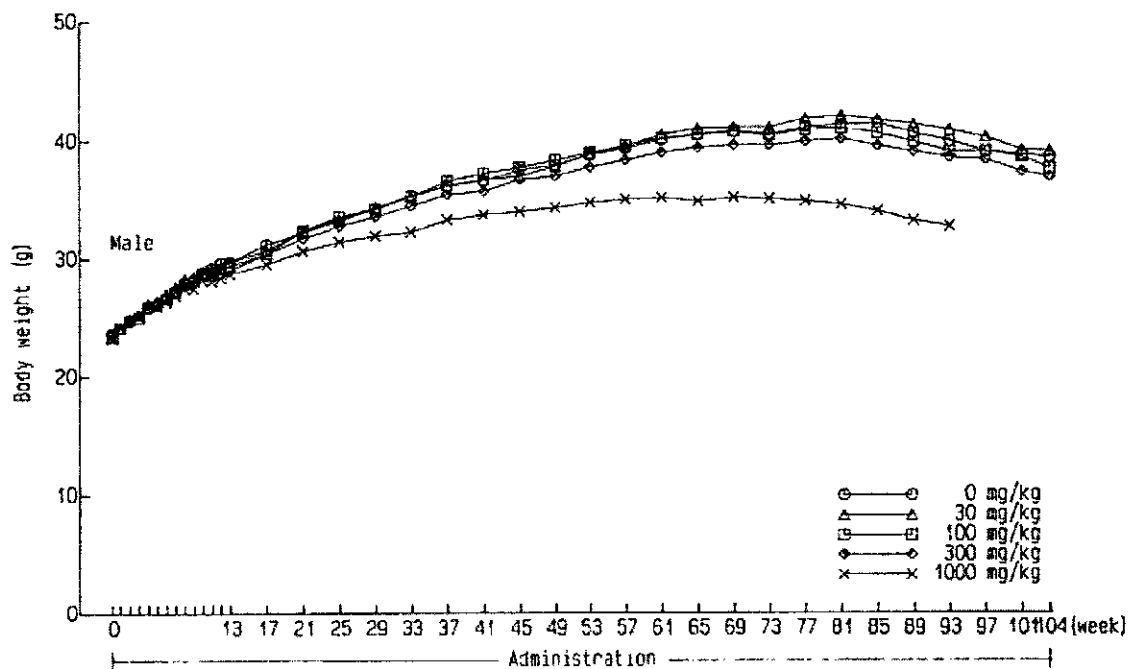
N: No remarkable change, -: Decrease, NA; not available

a): Necropsied after week 94 of administration (in week 95 of administration).

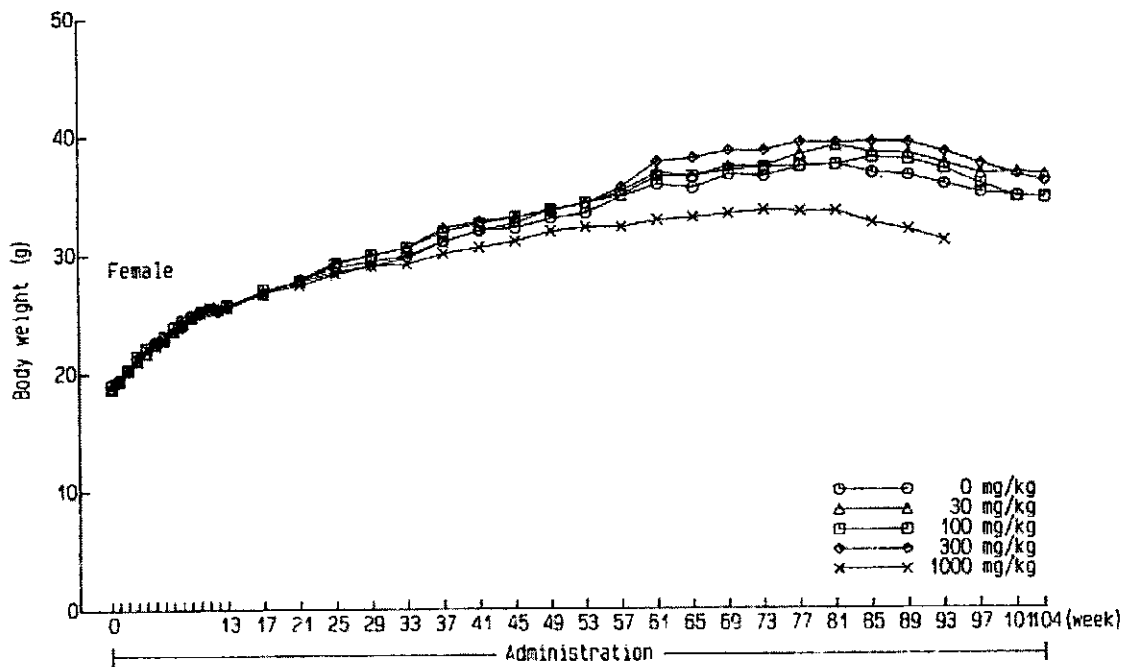
b): Comparison with the control mean value at week 94.

*: p<0.05 (significantly different from the control group)

Apparent effects of TAK-375 at 1000 mg/kg/day on body weight were apparent as early as Week 9 according to the Sponsor (see Sponsor's Figures below).



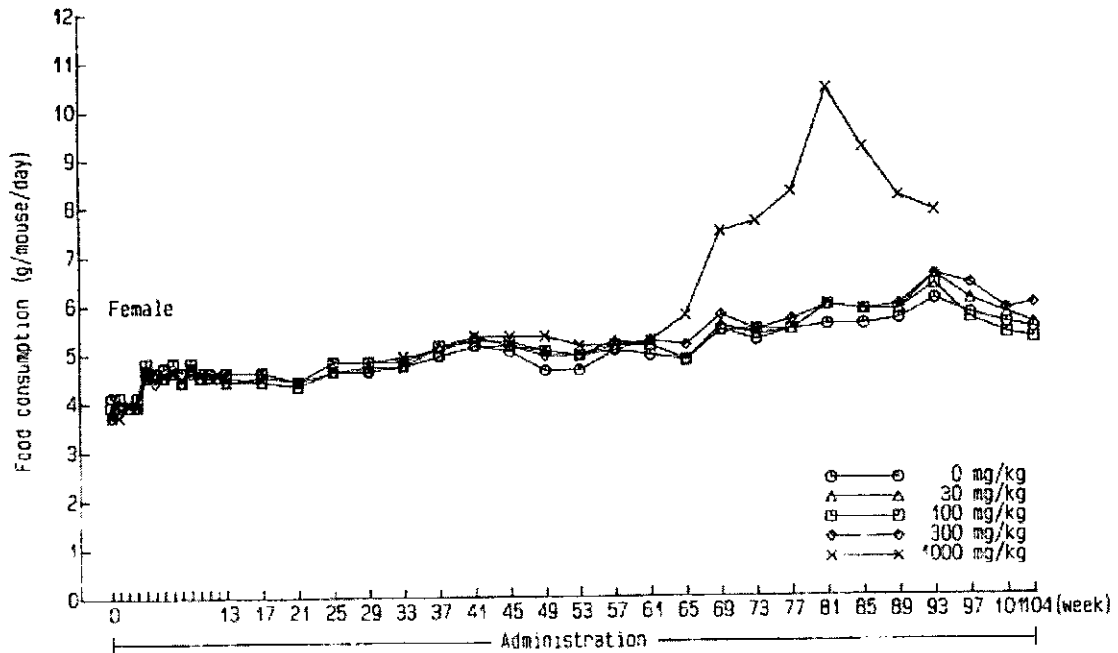
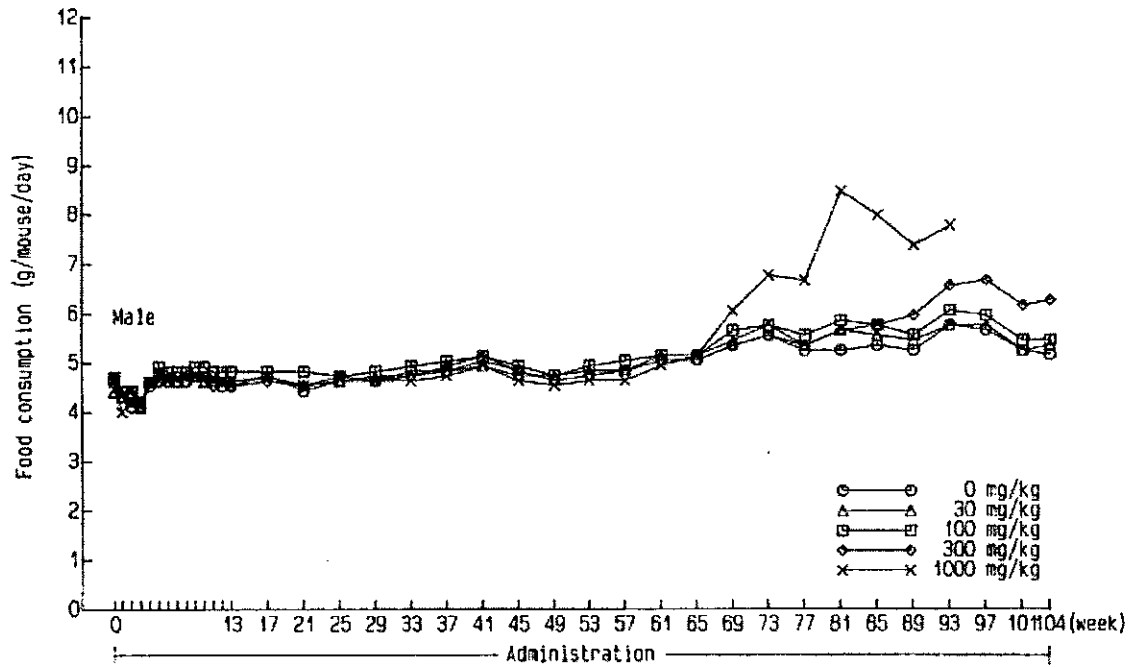
Female mice administered TAK-375 1000 mg/kg/day had a significant reduction in body weight, ↓15%, by the end of administration (94 weeks) when compared with control animals. This effect began to appear as early as 38 weeks after initiation of dosing according to the Sponsor (see Sponsor's Table 5, above and Figure below).



Food consumption:

Analysis of food consumption between treated groups and controls revealed a strong effect of TAK-375 treatment group such that in both male and female mice, the 1000 mg/kg/day dose level was associated with a significant increase in food consumption beginning approximately in the 65th week (see Sponsor's figures below).

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Peak food consumption appeared to occur at Week 81 for both sexes and was maximally 62% and 87% above control group levels (males and females, respectively). Some diminishment of this effect was noted after the peak response and a general decline was observed until the early termination of this group during after week 94. Male mice receiving 300 mg/kg/day TAK-375 demonstrated a statistically significant increase in food consumption beginning in week 87 (i.e.

in the 20th month of the study). No such consistent effect was noted in females at this dose level during the study. Sporadic instances of increased or decreased food consumption in comparison to control were noted in many of the treated groups, though the due to the inconsistent appearance of these fluctuations these are considered incidental.

Clinical Pathology:

Hematology

Significant alterations in hematologic parameters were observed in male and female mice chiefly at dose levels of ≥ 300 mg/kg/day and involved increases in mean cell hemoglobin and concentration (in males only) and platelets (both males and females). The 1000 mg/kg/day dose level was associated with increased hematologic changes and included increases in RBCs, hemoglobin, hematocrit, mean cell hemoglobin, mean cell hemoglobin concentration and mean cell volume (see Sponsor's Table below for details). A finding of increased mean cell hemoglobin in males administered 100 mg/kg/day was statistically significant though the effects ($\uparrow 1\%$ vs. control) did not worsen at the 300 mg/kg/day dose level and thus are of uncertain toxicologic significance.

Text Table 6. Summary of major hematological data

Sex	Males				Females			
	30	100	300	1000 ^{a)}	30	100	300	1000 ^{d)}
Dose (mg/kg/day)								
No. of animals used	44	41	41	25	39	34	37	24
RBC	N	N	N	N	N	N	N	+14%*
Hb	N	N	N	+12%*	N	N	N	+20%*
Ht	N	N	N	+5%*	N	N	N	+18%*
MCV	N	N	N	N	N	N	N	+2%*
MCH	N	+1%*	+1%*	+8%*	N	N	N	+5%*
MCHC	N	N	+3%*	+7%*	N	N	N	+3%*
Reticulocyte	N	N	N	+50%*	N	N	N	N
Platelet	N	N	+22%*	+47%*	N	N	+15%*	+45%*

Values in the table indicate change of percentage against the mean control value.

N: No remarkable change, +: Increase

a): Necropsied after week 94 of administration (in week 95 of administration).

*: $p \leq 0.05$ (significantly different from the control group)

Gross pathology:

Macroscopic examination of tissues collected from animals at necropsy indicated several apparent TAK-375-associated findings which appear to exhibit dose-relationship (Table 1 below). Among these were increases in the incidence of Harderian gland node in TAK-375-treated animals as compared with those treated with vehicle alone. The males at the 1000 mg/kg/day dose level appeared to have slightly fewer observations (13/55) relative to the other treated males (15-20/55) though this was still more than double the number of observations in control group animals (6). Liver nodes appeared to increase markedly from control with dosing in males ≥ 100 mg/kg/day and in females with dosing ≥ 300 mg/kg/day. All but one animal in the 1000 mg/kg/day dose level had evidence of liver nodules on gross inspection. Other notable dose-related findings were the increase in raised foci in the stomach of male and female mice given 1000 mg/kg/day until sacrifice and a mild increase in discoloration in

portions of the stomach which appeared to be observed at all dose levels but particularly at the 1000 mg/kg/day level.

Gross Pathology Findings (Combined Early Decedents with Terminal Sacrifice)

Dose	Male					Female				
	0	30	100	300	1000	0	30	100	300	1000
n	55	55	55	55	55	55	55	55	55	55
Harderian Gland										
Node	6	15	15	20	13	4	6	8	9	11
Liver										
Node	23	29	41	53	54	18	14	13	49	55
Stomach										
Raised focus	2	3	1	4	25	1	2	0	2	27
Discolored focus	2	4	5	6	9	1	5	3	3	11

The Sponsor's table with all gross findings which were observed in $\geq 5\%$ of at least a single TAK-375-treated group is reproduced below.

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Text Table 7. Incidence of major gross lesions

Sex	Male					Female				
	0	30	100	300	1000	0	30	100	300	1000
Dose (mg/kg/day)										
No. of animals used	55	55	55	55	55	55	55	55	55	55
Abdominal cavity										
excess fluid	3	3	3	2	3	7	8	9	7	1
Harderian gland										
node	6	15	15	20	13	4	6	8	9	11
Lymph node, nos ^{a)}										
large	3	1	1	3	0	8	12	13	13	0
Lymph node, mesenteric										
large	5	9	3	3	1	9	8	11	11	1
Lymph node, submandibular										
large	4	1	2	0	1	3	9	7	10	0
Liver										
node	23	29	41	53	54	18	14	13	49	55
focus, raised	4	1	2	4	3	0	1	3	6	1
focus, discolored	8	10	7	9	7	9	9	11	12	5
Lung (bronchus)										
node	13	11	15	17	12	6	7	12	7	5
Ovary										
cyst	-	-	-	-	-	1	2	7	2	1
cystic ovarian bursa	-	-	-	-	-	8	6	11	6	2
Preputial gland										
large	3	6	1	2	0	-	-	-	-	-
Skin										
node	4	3	1	0	1	1	5	6	2	1
excoriation	3	0	6	3	2	1	2	1	1	1
Spleen										
large	5	4	1	2	2	6	12	12	15	3
Stomach										
focus, raised	2	3	1	4	25	1	2	0	2	27
focus, discolored	2	4	5	6	9	1	5	3	3	11
Thoracic cavity										
excess fluid	1	1	2	0	0	4	3	4	7	0
Uterus										
node	-	-	-	-	-	7	4	1	3	3
cyst, endometrial	-	-	-	-	-	37	33	37	32	8

a): Lymph nodes other than the submandibular and mesenteric lymph nodes.

Additional significant gross findings which displayed a TAK-375-treatment effect included slightly increased incidence of large lymph nodes in female mice dosed with 30 mg/kg/day – 300 mg/kg/day TAK-375 compared with controls. The 1000 mg/kg/day TAK-375 dose group animals, both males and females, had fewer incidences of large lymph nodes at several sites (including mesenteric and submandibular) relative to their lower dose counterparts or the control animals. This observation is thought due to an increase of hepatic tumors and bodyweight loss in this group and thus is considered by the Sponsor to be an indirect effect of TAK-375 treatment. TAK-375 was associated with a decrease in several gross observations including a reduction in ovarian cysts compared with control animals (2/55 vs. 8/55) and a reduction in cystic masses in the uterus as well as a mild reduction in the observations of node in this tissue. This is believed by Sponsor to be due to an alteration in endocrine status and/or

poor condition due to liver tumor. No supportive evidence was presented within this study, however. Additionally, large spleen size was observed in females administered TAK-375 $\leq$ 300 mg/kg/day group more frequently than controls but the 1000 mg/kg/day dose level had fewer animals with this finding than controls. The Sponsor makes the argument once again that "it was attributable to decrease in the incidence of malignant lymphoma probably caused by suppressed body weight gain and/or severe development of liver tumor".

Incidence of Macroscopic Findings by Treatment Group

Dose	Males					Females				
	0	30	100	300	1000	0	30	100	300	1000
n	55	55	55	55	55	55	55	55	55	55
Lymph node										
NOS, large	3	1	1	3	0	8	12	13	13	0
Mesenteric, large	5	9	3	3	1	9	8	11	11	1
Submandibular, large	4	1	2	0	1	3	9	7	10	0
Ovary										
Cyst	-	-	-	-	-	8	6	11	6	2
Spleen										
Large	5	4	1	2	2	6	12	12	15	3
Uterus										
Node	-	-	-	-	-	7	4	1	3	3
Cyst, endometrial	-	-	-	-	-	37	33	37	32	8

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Histopathology:

Neoplastic:

Tumors within an organ system which are present at an incidence of 5% or greater (benign and malignant combined) are presented in Sponsor's Table below.

Microscopic Lesions for Neoplastic Lesions: All Animals

Sex Dose (mg/kg/day) No. of animals used	Male					Female				
	0	30	100	300	1000	0	30	100	300	1000
Hemolymphoreticular (all sites)										
Lymphoma, malignant	4	5	5	6	2	13	17	19	21	4*
Sarcoma, histiocytic	4	4	5	1	3	5	9	3	5	6
Harderian gland										
Adenoma	6	15 ^{a†}	14 ^{a†}	16 ^{a†}	11	3 [‡]	3	10 ^{a†}	10 ^{a†}	9 [‡]
		0.0158*	0.0232	0.0084		0.0018*		0.0119	0.0141	0.0130
Adenocarcinoma	1	1	1	2	1	1	2	0	0	0
Liver										
Adenoma, hepatocellular	20 [‡]	24	32 ^{a†}	50 ^{a†}	54 ^{a†}	10 [‡]	14	14	52 ^{a†}	55 ^{a†}
	0.0000		0.0213	0.0000	0.0000	0.0000			0.0000	0.0000
Carcinoma, hepatocellular	9 [‡]	6	18 ^{a†}	26 ^{a†}	50 ^{a†}	4 [‡]	3	3	11 ^{a†}	51 ^{a†}
	0.0000		0.0201	0.0003	0.0000	0.0000			0.0185	0.0000
Hepatoblastoma	0 [‡]	0	1	3 [‡]	10 ^{a†}	0 [‡]	0	0	1	3 [‡]
	0.0000			0.0416	0.0003	0.0016				0.0036
Hemangioma	2	4	1	0	0	0	1	1	1	0
Hemangiosarcoma	0	4 [‡]	4 [‡]	0	0	3	3	1	0	0
Lung (bronchus)										
Adenoma, bronchiolo-alveolar	7 [‡]	6	10	15 ^{a†}	8 [‡]	2	4	5	2	3
	0.0137			0.0301	0.0374					
Adenocarcinoma	5	2	1	0 [‡]	0 [‡]	2	0	5	2	2
Adenoma, bronchiolo-alveolar + Adenocarcinoma	12	8	11	15	8	4	4	10 [‡]	4	5
								0.0363		
Pituitary										
Adenoma, anterior	0	0	0	0	0	3	3	5	2	0
Skin										
Sarcoma, spindle cell	0	0	0	0	0	0	1	4 [‡]	1	1
Spleen										
Hemangioma	2	0	2	1	1	2	1	0	1	0
			(n=11) ^{a†}							
Hemangiosarcoma	0	4 [‡]	0	0	0	2	4	1	0	0
Uterus										
Polyp, endometrial stromal	-	-	-	-	-	1	3	0	0	0

- a): Peto's positive trend test p- value of pooled tumor incidence for all groups
- b): Pairwise comparison of pooled tumor between the dosage group and the control group
- c): Effective number
- *: p<0.05 (significantly different from the control group, Fisher's exact test)
- †: p<0.05 (significantly different from the control group, Peto's test)
- ‡: p<0.05 (significantly different from the control group, Peto's positive trend test)

Clear TAK-375-associated tumors which exhibited dose-dependence in this species were found in the liver and comprised hepatocellular adenoma (males and females at ≥300 mg/kg/day), carcinoma (males ≥300 mg/kg/day; females at 1000 mg/kg/day) and hepatoblastoma though a trend test indicated the hepatoblastoma was significant only in male mice (males ≥ 300 mg/kg/day) as shown in the Table below. Note that the trend test and pairwise statistical analysis conducted by Dr. Joan Buenconsejo utilized different levels of significance for common tumors (p <0.005 and p <0.01, respectively) defined as being observed in ≥ 1% of control animals versus rare tumors (p <0.025 and p <0.05, respectively) which were below 1% incidence in controls. Analysis of the combined incidence of hepatocellular adenoma and carcinoma reduced the dose level at which a significant difference from control animals as found in males to the 100 mg/kg/day dose level while in females this was unchanged from the separate analysis of adenoma data, i.e. 300 mg/kg/day. The other organ for which there

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appeared the possibility of an association between TAK-375 and tumor development in mice was the Harderian gland. According to Sponsor's analysis the increased incidence of Harderian gland adenoma was statistically significant by trend test in female mice but not in males, though individual pairwise comparisons in males did identify group incidences which were different from control (30 – 300 mg/kg/day groups but not 1000 mg/kg/day). The FDA statistical review did not identify this positive trend in either male or female mice using more stringent p-value cutoff scores as used for common tumors. The p-value cutoff used for trend significance was $p < 0.005$ and it should be noted that the actual p-value obtained was $p = .0164$ for female. Although the incidence of Harderian gland adenoma was higher in all treatment groups when compared with the respective control groups and appeared to be well above NTP historical controls, dose-dependent increase in tumor incidence was not observed over a >30-fold increase in TAK-375 dose.

**Apparent TAK-375-Related Neoplasms in 104-Week Mouse Bioassay
(Combined Early and Terminal Sacrifice)**

	TAK-375 Dose (mg/kg/day; oral gavage)											
	Males						Females					
	0	30	100	300	1000 [†]	Trend [#]	0	30	100	300	1000 [†]	Trend [#]
n =	55	55	55	55	55		55	55	55	55	55	
LIVER												
<i>Adenoma</i>												
Incidence	20	24	32	50*	54*	Yes	10	14	14	52*	55*	Yes
%	36	44	58	91	98		18	25	25	95	100	
Historical Mean (max) [†]	34.9% (max: 36.7%)						16.9% (max: 22%)					
Historical Mean(max) [*]	43.3 ± 9.9% (max: 53%)						35.0 ± 8.7% (max 40%)					
<i>Carcinoma</i>												
Incidence	9	6	18	26*	50*	Yes	4	3	3	11	51*	Yes
%	16	11	33	47	91		7	5	5	20	93	
Historical Mean (max) [†]	17.1% (max: 20.0%)						2.7% (max: 7.3%)					
Historical Mean(max) [*]	31.6 ± 10.1% (max: 42%)						23 ± 6.2% (max: 28%)					
<i>Hepatoblastoma</i>												
Incidence	0	0	1	3*	10*	Yes	0	0	0	1	3	No
%	0	0	2	5	18		0	0	0	2	5	
Historical Mean (max) [†]												
Historical Mean(max) [*]	1.5 ± 1% (max: 2%)						0.5 ± 1% (max: 2%)					
<i>Combined liver tumors (adenoma and carcinoma)</i>												
Incidence	25	28	40*	54*	54*	Yes	13	16	17	53*	55*	Yes
%	45	51	73	98	98		24	29	31	96	100	
<i>Combined liver tumors (adenoma, carcinoma and hepatoblastoma) – same incidence as above</i>												
Incidence	25	28	40*	54*	54*	Yes	13	16	17	53*	55*	Yes
%	45	51	73	98	98		24	29	31	96	100	

	TAK-375 Dose (mg/kg/day; oral gavage)												
	Males						Females						
	0	30	100	300	1000	Trend ^a	0	30	100	300	1000	Trend ^a	
Hepatic adenomas													
Incidence	6	15	14	16	11	No	3	3	10	10	9	No	
Historical mean (max)	11	27	25	29	20		5	5	18	18	16		
Biological mean (max)	9.2% (max: 11.7%)						9.3% (max: 16.7%)						
Biological mean (max)	6.6% (max: 8.2%)						3.5% (max: 10%)						
Adipose gland adenomas													
Incidence	1	1	1	2	1	No	1	2	0	0	0	No	
Historical mean (max)	2	2	2	4	2		2	4	0	0	0		
Biological mean (max)	1.7% (max: 3.3%)						0.4% (max: 1.8%)						
Biological mean (max)	0% (max: 0%)						3.5% (max: 10%)						
mice at 1000 mg/kg/day dose level were sacrificed during Week 95 (all other groups Week 104) from Lakota 1 B66. Liver: total number of tissues examined: 175 (♂), 225 (♀) from NTP historical database. H&E: 16 (♂) and 16 (♀) of 16 tissues. All sites: total number of animals examined = 200.													
^a Significantly different from control group (p < 0.05 [common tumors] or p < 0.025 [rare tumors]) or significantly different from control group (p < 0.05 [common tumors] or p < 0.025 [rare tumors]).													

Of interest, according to the executive summary of the statistical analysis of the data by Dr. Joan Buenconsejo, removal of the 1000 mg/kg/day dose level from the trend analysis results in the same findings with the exception that hepatocellular carcinoma in females becomes non-significant across the remaining doses.

Number of Tumors and Tumor-Bearing Animals (Combined Early and Terminal Sacrifice)

Sex	Male					Female				
	0	30	100	300	1000	0	30	100	300	1000
Dose (mg/kg/day)										
No. of animals used	55	55	55	55	55	55	55	55	55	55
No. of benign tumors	46	71	87	241	283	29	41	52	245	273
No. of malignant tumors	26	33	48	51	143	34	51	40	50	172
No. of tumors	72	104	135	292	426	63	92	92	295	445
No. of benign tumor bearers	30	33	44	52	54	24	27	34	52	55
No. of malignant tumor bearers	23	23	30	33	52	27	36	32	37	51
No. of multiple tumor bearers	16	27	39	53	54	17	23	22	53	54
No. of tumor bearing animals	43	39	50	55	54	39	47	48	55	55

Non-neoplastic: The following histopathologic observations were noted to occur more frequently in TAK-375-treated mice compared with their respective control groups. Note that no statistical analysis was conducted by Sponsor.

Incidence of Non-Neoplastic Findings

	TAK-375 Dose (mg/kg/day)									
	Males (n = 55/group)					Females (n = 55/group)				
	0	30	100	300	1000 [†]	0	30	100	300	1000 [†]
ADRENAL										
Extramedullary Hematopoiesis	0	0	0	0	0	1	2	2	2	3
Hypertrophy, cortical cell, focal	1	7	3	10	42	6	10	11	14	51
EPIDIDYMS										
Cell infiltration, lymphocytic	6	10	6	4	21	-	-	-	-	-
KIDNEY										
Pigmentation, tubular cell	0	1	2	9	17	9	9	1	2	22
Glomerulosclerosis	5	5	4	6	23	6	2	0	3	5
LYMPH NODE										
Mesenteric, atrophy	2	2	2	3	6	1	9	1	1	12
Submandibular, atrophy	2	0	2	2	1	1	1	1	0	5
LIVER										
Necrosis, single cell	2	0	4	2	17	1	1	1	0	2
Necrosis, focal	2	4	7	4	3	0	0	0	6	4
Necrosis, centrilobular	0	0	1	1	3	1	1	0	1	1
Pigmentation, sinusoidal lining cell	0	0	2	2	44	0	1	1	0	31
Hypertrophy, hepatocyte, central	0	3	3	27	5	0	1	1	1	0
Hypertrophy, hepatocyte, periportal	0	0	0	3	15	0	1	0	0	16
Altered cell focus, eosinophilic	6	20	19	33	27	3	1	9	31	25
OVARY										
Atrophy	-	-	-	-	-	18	12	18	23	55
SPLEEN										
Atrophy, lymphoid	4	0	1	1	1	0	0	2	2	7
Hematopoiesis, increased	18	17	22	22	46	24	36	24	34	48
STOMACH										

Erosion, glandular stomach	2	4	4	3	5	1	4	3	3	7
Hyperplasia, squamous, focal, forestomach	3	3	1	3	30	1	1	1	1	29
Cell infiltration, inflammatory, forestomach	1	1	1	1	24	0	1	0	0	16
SEMINAL VESICLE										
Atrophy	1	4	9	4	34	-	-	-	-	-
THYMUS										
Atrophy	23	24	28	29	43	17	18	22	16	46
THYROID										
Pigmentation, follicular cell	0	0	0	28	49	0	1	3	31	43
Hypertrophy, follicular cell	0	0	1	2	33	0	0	1	5	46
UTERUS										
Atrophy	-	-	-	-	-	3	10	8	13	54
VAGINA										
Atrophy	-	-	-	-	-	4	11	8	16	50

Toxicokinetics:

TAK-375: Exposure (AUC_{0-24}) was somewhat greater than dose-proportional across doses in males and females with clear evidence of reduced exposure over the course of the study, primarily by Week 13 with dosing ≥ 300 mg/kg/day in males. Although mild reduction in exposure over time occurred in females at 30 mg/kg/day, a much greater reduction in exposure was noted with dosing ≥ 100 mg/kg/day. Males and females had broadly similar exposure to TAK-375.

M-II: Exposure (AUC_{0-24}) was much greater than dose-proportional up to 300 mg/kg/day in males while at 1000 mg/kg/day exposure was lower than that observed at 300 mg/kg/day. In females, exposure showed dose-dependence and was broadly dose-proportional overall though exposure in animals of the high dose level was lower on a dose-normalized basis than those in the 100 and 300 mg/kg/day treatment group. Higher exposure was observed in males relative to females with dose levels ≥ 100 mg/kg/day.

Appears This Way
On Original

TAK-375

Analyte	Parameter	Stage	Sex Dose (mg/kg/day)	Male				Female			
				30	100	300	1000	30	100	300	1000
TAK-375	T _{max} (h)	1st		0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5
		13 w		0.3	0.3	0.5	1.0	0.3	0.3	0.3	1.0
		52 w		0.3	0.3	0.5	0.5	0.3	0.5	0.3	1.0
	C _{max} (ng/mL)	1st		1789	15809	26065	54571	1667	15289	32493	85567
		13 w		2366	12461	16278	11623	2013	9856	11955	11813
		52 w		2015	10052	13327	12558	1444	6185	11848	14374
	AUC _{0-24h} (ng·h/mL)	1st		867	12688	133290	578872	2882	15655	127662	323636
		13 w		971	12412	62874	56305	766	9524	31802	43709
		52 w		967	10138	50425	55003	722	7773	21061	48829

Metabolites of TAK-375

Analyte	Parameter	Stage	Sex Dose (mg/kg/day)	Male				Female			
				30	100	300	1000	30	100	300	1000
M-I	T _{max} (h)	1st		0.3	0.3	0.5	1.0	0.3	0.3	0.3	0.5
		13 w		0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.3
		52 w		0.3	0.3	0.5	0.5	0.3	0.3	0.3	0.3
	C _{max} (ng/mL)	1st		1768	4365	6440	9229	2783	6404	9820	15872
		13 w		1812	4377	6386	7716	4599	10262	15918	18909
		52 w		1481	3636	5816	7811	3627	8445	17060	16839
	AUC _{0-24h} (ng·h/mL)	1st		1490	5741	22241	86267	1757	7856	35846	68267
		13 w		1334	5385	25220	37924	1974	9735	31055	60134
		52 w		1069	5085	19623	35953	2193	9203	34979	62598
M-II	T _{max} (h)	1st		0.5	1.0	3.0	6.0	0.3	1.0	1.0	3.0
		13 w		0.3	0.5	1.0	1.0	0.3	0.5	1.0	1.0
		52 w		0.3	1.0	1.0	3.0	0.3	0.5	0.5	1.0
	C _{max} (ng/mL)	1st		1069	4307	7220	13080	876	3470	4688	7460
		13 w		1264	5188	6365	4079	849	2296	2949	2553
		52 w		936	3343	4485	3425	493	1608	1928	3769
	AUC _{0-24h} (ng·h/mL)	1st		1149	9961	77841	192831	688	6660	44233	77841
		13 w		1039	10645	58425	39775	459	3828	13324	16122
		52 w		614	7203	37761	28427	285	2789	7996	14496
M-III	T _{max} (h)	1st		0.3	0.5	1.0	6.0	0.3	0.5	0.5	3.0
		13 w		0.3	0.5	0.5	1.0	0.3	0.5	0.5	0.5
		52 w		0.3	0.5	0.5	0.5	0.3	0.5	0.5	1.0
	C _{max} (ng/mL)	1st		5727	18230	27940	39762	5997	17251	24396	37754
		13 w		8133	22707	19484	13631	7627	20559	17822	16184
		52 w		9313	16873	20880	15041	6125	15990	20545	22014
	AUC _{0-24h} (ng·h/mL)	1st		5576	35840	214173	606332	4852	31849	230436	401961
		13 w		5938	35427	117545	92177	4838	32138	81497	106438
		52 w		5117	31902	109549	89069	4173	29729	80833	119109

	1st	1.0	1.0	3.0	6.0	0.5	1.0	3.0	6.0
T _{max} (h)	13 w	0.5	1.0	3.0	1.0	0.5	1.0	3.0	3.0
	52 w	0.5	1.0	3.0	3.0	0.5	1.0	1.0	1.0

M-IV C _{max} (ng/mL)	1st	2284	3199	3516	3136	1345	1919	2410	2109
	13 w	2050	4261	3588	1968	1251	2495	2774	2352
	52 w	1831	3960	2995	2891	827	1867	1852	2563

AUC _{0-24h} (ng·h/mL)	1st	4612	11735	38199	50942	2322	6386	25608	32540
	13 w	4365	14668	42090	26360	1918	5616	21136	24755
	52 w	3669	12544	33352	23642	1319	5984	16921	22386

Comparison of TAK-375 and M-II Metabolite Exposures at Mouse Tumor NOAEL versus Expected Clinical Exposure with TAK-375 at 8 mg/day

Tumor/Type	SEX	NOAEL DOSE (mg/kg/day)	[TAK-375] (ng·h/mL)	HUMAN MOS†	[M-II] (ng·h/mL)	HUMAN MOS†
LIVER						
Adenoma	M	100	10,138	1,079-fold	7,203	30-fold
	F	100	7,773	827-fold	2,789	12-fold
Carcinoma	M	100	10,138	1,079-fold	7,203	30-fold
	F	300	21,061	2,241-fold	7,996	33-fold
Adenoma + Carcinoma	M	30	967	103-fold	614	3-fold
	F	300	7,773	827-fold	2,789	12-fold
Hepatoblastoma	M	100	10,138	1,079-fold	7203	30-fold
	F	>1000	48,829	5,195-fold	14,496	> 60-fold

†MOS – Margin of Safety; based on results from study 01-01-TL-375-03; elderly administered 16 mg with a extrapolation to 8 mg based on linear kinetics within this dose range. Estimated AUC_{0-24h} TAK-375: 9.4 ng·h/mL; M-II: 241.3 ng·h/mL.

Summary of individual study findings:

Adequacy of carcinogenicity study and appropriateness of test model:

The mouse is a common model for carcinogenicity studies and the B6C3F1 strain is an appropriate strain for use. In this study TAK-375 was administered orally as an oral gavage with drug dissolved in a vehicle solution of 0.5% methylcellulose. The oral route is the intended route in humans, though TAK-375 will be administered in tablet form. In the present study mice were dosed daily, a frequency which may be expected in humans. Dosing solutions were demonstrated to be stable and uniform in a separate study to support the frequency and method of drug preparation and use. The TAK-375 metabolite profile is qualitatively similar between mice and humans (see Toxicokinetic section of this review) though in man the M-II metabolite, which is active, circulates in quantitatively much greater concentration relative to TAK-375 (87:1) than in mice (~1:1 ♂; ~1:3 ♀). Nevertheless, in the present study the low dose of TAK-375 produced exposure to the M-II metabolite that represents approximately 2.6 and 1.1-fold, (males and female mice, respectively) the clinical exposure to M-II when taken as an 8 mg/day dose. The high dose administered to mice represents a 118- and 60-fold exposure above the expected clinical exposure to the M-II metabolite. Therefore, the present study appears to provide adequate exposure to both parent and the active metabolite such that relative metabolism differences between mouse and human

should not affect the ability to use the results of this study as part of the human carcinogenicity risk assessment.

Adequacy of dosing was of particular concern for the Executive Carcinogenicity Assessment Committee which was unable to concur with the proposed dosing used in the study due to the potential for 1000 mg/kg/day to exceed the maximum tolerated dose (MTD) and the 300 mg/kg/day dose level to be too far below the MTD. An assessment of the adequacy of dosing used in present study reveals the high dose level of 1000 mg/kg/day is indeed above the MTD in both male and female mice. This dose level was associated with excessive weight loss in the face of a significant increase in food consumption relative to controls and poor survival such that early termination of males and females in this group occurred during week 95 of the study. The early termination of this group as well as the significant reduction in body weight conceivably could confound interpretation of the study data as both factors could act to alter the types and frequency of tumors identified relative to lower dose groups due to mechanisms which are not directly related to TAK-375. Though this remains a possibility, the apparent TAK-375-related tumors identified at the 1000 mg/kg/day level occurred in the 300 mg/kg/day dose levels as well and this dose was not associated with poor survival, though males demonstrated a slight but significant reduction in bodyweight gain when compared with controls. Taken together, while 1000 mg/kg/day appeared to be above the MTD for males and females, the 300 mg/kg/day dose level was probably near the MTD at least in males.

Evaluation of tumor findings:

Tumors apparent after up to 104 weeks of oral administration of TAK-375 included hepatic adenoma and carcinoma in males and females, and hepatoblastoma in males only. The incidence of Harderian gland adenomas appeared to be elevated in all TAK-375 groups relative to control animals but this did not reach statistical significance by trend analysis and though the incidence was above historical control levels in 104-week studies no dose-dependence was demonstrated over a 30-fold dose range.

Males appeared more sensitive to the development of hepatic tumors demonstrating an increased incidence of adenoma and carcinoma with dosing levels ≥ 300 mg/kg/day while in females hepatic adenoma was significantly increased at 300 mg/kg/day and carcinoma reached statistical significance only at 1000 mg/kg/day. Combining hepatocellular adenoma and carcinoma incidence reduced the dose level at which males demonstrated an increased tumor incidence to 100 mg/kg/day while this analysis did not alter the liver NOAEL in females. In addition to hepatic adenoma and carcinoma, males also developed hepatoblastoma starting at 300 mg/kg/day, while no significant increase in the incidence of hepatoblastoma was detected in females.

Due to the concerns over the 1000 mg/kg/day dose level, trend analysis of the remaining groups after exclusion of the high dose level revealed no change in tumors reaching statistical significance in males while in females only hepatic adenoma exhibited a significant trend.

Hepatic Tumors Reaching Statistical Significance

	<i>Including 1000 mg/kg/day group</i>	<i>Excluding 1000 mg/kg/day group</i>
	Hepatic adenoma	No change
	Hepatic carcinoma	
	Hepatoblastoma	
	Hepatic adenoma	Hepatic adenoma
	Hepatic carcinoma	

The greater sensitivity demonstrated in male mice for hepatic tumor formation with increased incidence of tumors at lower dose levels may be related to the observation of higher plasma concentrations of TAK-375 and M-II when compared with female mice. Nevertheless, nearly all animals with dosing ≥ 300 mg/kg/day developed adenomas (109/110 males and females combined) and nearly all animals (101/110 combined) dosed with 1000 mg/kg/day developed hepatic carcinoma. Thus, development of hepatic tumors occurred across gender in a dose-dependent manner. The latency of tumor development is unclear though it is assumed that the greater mortality observed in high dose males by Week 70 and in females by Week 76 was due to the unsustainable burden of liver tumors. The Sponsor states that hepatic carcinoma and hepatoblastoma was found to metastasize to lung and more infrequently the heart.

A previous 13-week dose range-finding study in mice revealed evidence of liver enzyme induction as measured by aminopyrine-N-demethylase activity with dose levels of ≥ 100 mg/kg/day while a 1-week study with oral administration of TAK-375 in mice showed induction of similar CYP enzymes induced as has been observed with phenobarbital. Phenobarbital has been demonstrated by previous investigation (McClain, 1995) to produce a dose-related increase in hepatic carcinoma and hepatoblastoma in B6C3F1 mice along with hepatic enzyme induction while others (Harada et al., 1999) have shown phenobarbital more broadly produces hepatic tumors in rodents. This response has not been considered to be generalizable to humans based on epidemiological studies of this class of drugs (i.e. hepatic enzyme inducers) in clinical populations (McLean et al., 1990; McClain, 1995; Whysner et al., 1996).

Administration of TAK-375 was associated with an apparent increase in Harderian gland adenoma though this did not reach statistical significance by trend analysis using stringent cut-off p-values for common tumors. Nevertheless, both treated males and females appeared to have an incidence well above control animals and historical controls, though little dose-dependency was shown and in both males and females the high dose was associated with a slightly lower incidence of these tumors. Caution must be taken when assessing this lower incidence, however, due to the early termination of the high dose mice, 9 weeks prior to all other groups which could explain the difference in frequency if these tumors are slowly developing. Adenocarcinoma of the Harderian gland was not observed to increase and no pre-neoplastic lesions were noted with histopathologic assessment of this tissue.

Tumors of the Harderian gland are generally not considered to be extrapolatable to the human as there is no comparable organ in the human. Nevertheless, the Harderian gland is known to produce and secrete melatonin and contains melatonin receptors. Additionally, melatonin has

been shown to stimulate the Harderian gland in the rat (Dhanaraj and Joshi, 1995) and hamster (Coto-Montes et al., 1993) therefore tumor development could be an exaggerated pharmacologic response to over-stimulation of the organ.

Despite the likelihood of a non-genotoxic mechanism underlying the generation of the observed tumors, these epigenetic explanations may not be required as TAK-375 and/or its metabolites have demonstrated evidence of genotoxicity in an *in vitro* chromosomal aberration assay both with and in the absence of metabolic activation. Though the results of several additional *in vitro* studies and *in vivo* micronucleus assays in mouse and rat were judged negative, a subsequent *in vivo/in vitro* Unscheduled DNA Synthesis assay conducted in rat liver tissue was considered positive by the reviewer, Dr. Aisar Atrakchi (see GENETIC TOXICOLOGY section). Therefore the potential remains that the tumors associated with TAK-375 exposure in the 2-year mouse bioassay are due to a direct genotoxic effect of the parent drug and/or a metabolite.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: B6C3F1 mice were administered vehicle or TAK-375 with dose levels of 30, 100, 300 or 1000 mg/kg/day by oral gavage for up to 104 weeks. Separate animals were used for toxicokinetic analysis of TAK-375 and metabolites up through Week 52 of the study. The high dose was clearly in excess of the MTD as excessive mortality was observed and requiring the early termination of this dose level in both males and females after 94 weeks of drug administration. Body weight was significantly decreased in animals at this dose level when compared with controls and body weight gain was decreased in 300 mg/kg/day group males but not females. Administration of TAK-375 was associated with the development of hepatic tumors, including hepatadenoma, hepatocarcinoma in males and females and hepatoblastoma in males. Exclusion of the 1000 mg/kg/day dose level data did not change the tumor observation in males though in females hepatocarcinoma became non-significant. The NOAEL for liver tumors was over 800-fold higher than the expected human exposure to TAK-375 at the therapeutic dose but was only 12-fold the expected exposure to the active metabolite M-II in humans. When hepatic tumors were combined in the statistical analysis, however, the dose level at which significance was reached was reduced and corresponds to a NOAEL exposure which provides a 103-fold margin of safety for TAK-375 and 3-fold margin of safety for M-II above the expected human exposure. The Sponsor argues that the liver tumors are due to hepatic enzyme induction which has been demonstrated to be associated with liver tumors in rodents and would not be generalizable to humans due to the absence of this linkage based on retrospective study. Histologic examination of hepatic tissue in this study indicated evidence of hepatocellular hypertrophy though this was not a finding in the majority of animals at the time of death or sacrifice.

Conclusions and Recommendations: The MTD for oral administration of TAK-375 was reached and exceeded in this mouse carcinogenicity assay. Dose-dependent increases in hepatic tumors are likely to be due to the demonstrated ability of TAK-375 to induce hepatic metabolizing enzymes. If true, this is not believed to indicate risk to humans. Nevertheless, it is noted that TAK-375 demonstrated positive findings in several genetic toxicology assays which could indicate a tumor risk that would be generalizable to humans.

Reviewer Signature Adam M. Wasserman, Ph.D.Supervisor Signature R. Daniel Mellon, Ph.D. concurrence: Yes X No **REFERENCES**

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Study title: Twenty-four month oral gavage carcinogenicity study of TAK-375 in rats**Key study findings:**

- Vehicle (0.5 w/v% methylcellulose, dual controls) or TAK-375 in a dose of 15, 60, 250 or 1000 mg/kg/day was administered by oral gavage to $\bar{m}$ CD(SD)IGS rats (n=60/sex/group) for up to 104 weeks. Satellite groups were used for toxicokinetics (n=10/sex/group) and consisted of rats administered 15, 60, 250 or 1000 mg/kg/day for 52 weeks with toxicokinetic analysis of TAK-375 and 4 metabolites determined on Day 1 and during Weeks 26 and 52.
- Excessive mortality was observed in the 1000 mg/kg/day dose level in female rats and required early termination of this group, during Week 94, an action which was not discussed with the Agency prior to its execution. Survival rate at end of study was between 28.3% (low dose females) – 57% (high-mid dose males) for all other groups. Death associated with dose levels of $\leq$ 250 mg/kg/day was considered due to the development of pituitary tumor while those occurring in the 1000 mg/kg/day groups were considered to be due largely to hepatic tumor or unspecified test article-related toxicity which was defined by death shortly after dosing with clinical signs of lateral or prone position. These latter deaths tended to occur earlier in the study.

- Administration of TAK-375 dosing of ≤ 60 mg/kg/day produced transient suppression of body weight during the first half of the study which were not observed by study termination. In contrast, dosing ≥ 250 mg/kg/day produced decreased weight at end of study in both male and female rats compared with controls. Suppression of body weight in males and females in the high dose group could be seen as early as Week 1 in males and Week 2 in females with a body weight plateau reached at approximately Week 17 for both sexes. For males of the 250 and 1000 mg/kg/day group body weight was $\downarrow 10\%$ and $\downarrow 39\%$, respectively compared with controls at the end of study. Body weight gain over the course of the study was decreased significantly only in the 1000 mg/kg/day group ($\downarrow 53\%$). For females of the 250 and 1000 mg/kg/day group body weight was $\downarrow 19\%$ and $\downarrow 44\%$ (94th week) compared with controls. Body weight gain in females was reduced by 29% at the 250 mg/kg/day dose level over 104 weeks. The body weight parameter for high dose females was not analyzed due to the early termination of this group. Food consumption was not significantly affected with oral administration of TAK-375.
- Hematologic changes were not observed (males) or were very mild (females) with TAK-375 dose levels ≤ 250 mg/kg/day. Administration of TAK-375 at the 1000 mg/kg/day dose level produced an increase in red blood cells (males), decreased hemoglobin and hematocrit (females), decreased mean cell volume, decreased mean cell hemoglobin but increased mean cell hemoglobin concentration, decreased reticulocytes (females), increased white blood cell count (females) and platelets.
- Administration of TAK-375 with dose groups of 0, 15, 60, 250 or 1000 mg/kg/day to rats by oral gavage for 104 weeks was associated with the development of hepatic adenoma (≥ 250 mg/kg/day) and carcinoma (at 1000 mg/kg/day) in males (adenoma: 0/120, 7/60, 7/60, 15/60* and 47/60*; carcinoma: 0/120, 0/60, 0/60, 0/60 and 38/60*) and adenoma (≥ 60 mg/kg/day) and carcinoma (at 1000 mg/kg/day) in females (adenoma: 2/120, 0/60, 8/60*, 22/60* and 49/60*; carcinoma: 0/120, 0/60, 1/60, 1/60 and 20/60*) as well as Leydig cell tumors in males at the highest dose level (3/120, 1/60, 0/60, 7/60, 46/60*). Combining the incidence of hepatocellular tumors in either males or females did not lower the threshold dose at which significant difference from control in tumor development was observed. The high dose used in the study appeared to exceed the MTD in both males and females as evidenced by the notable reduction in body weights in at this dose level relative to controls and the excessive mortality in females in the 1000 mg/kg/day group which required early termination during Week 94 of the study.
- Non-neoplastic changes observed with TAK-375 dosing which demonstrated evidence of a dose-dependent relationship to treatment in the liver included necrosis, hemorrhage, fibrosis, hypertrophy, altered cell focus and bile duct cystic hyperplasia and bile duct cell proliferation and oval cell proliferation. Changes in the testes included seminiferous tubule atrophy and Leydig cell hyperplasia while other changes in the male sex organs observed was prostatic atrophy. Other non-neoplastic findings observed with microscopic analysis included adrenal cortical cell vacuolation and hypertrophy, small follicular cells of the thyroid, and pigmentation noted in many organs including the central nervous system (cerebral cortex, cerebellum and spinal cord), glands (extraorbital lacrimal gland, thyroid and salivary glands), kidney and liver which may reflect test article material or breakdown product according to the Sponsor.

- The rat liver tumor NOAEL was determined to be 60 mg/kg/day in males and 15 mg/kg/day in females and corresponded to plasma levels of TAK-375 of 13,432 ng•h/mL and 4,434 ng•h/mL in male and female rats, respectively. This represents a 1,429-fold and 472-fold margin of safety over expected human therapeutic concentrations of TAK-375 using male and female rat values, respectively. However, the calculated safety margins for the active metabolite M-II are significantly lower when considering typical human exposure to this metabolite at the proposed clinical dose; approximately 13-fold using data from males and 16-fold from females. The Leydig cell tumor NOAEL is 250 mg/kg/day which corresponds to a TAK-375 plasma concentration of 42,033 ng•h/mL, providing a nearly 4,472-fold margin of safety when considering exposure to the parent compound in humans. The plasma concentration of the M-II metabolite at the NOAEL, however, only provides a 14-fold margin of safety over the expected human exposure to this metabolite.
- Extrapolation of these findings to the human is uncertain as xenobiotics such as TAK-375 which promote hepatic enzyme induction are known to produce hepatic adenomas and carcinomas in the mouse and adenomas in the rat. The present study also found evidence of hepatic carcinogenicity which is a more unusual finding with hepatic enzyme inducers though this has been reported in the past. Another potential epigenetic explanation for the hepatic tumors is the TAK-375-induced increase in dehydroepiandrosterone (DHEA) which is known to be a peroxisome proliferator and can promote as well as directly underlie the development of hepatic tumors. A separate study of 4-weeks duration of TAK-375 at doses used in this carcinogenicity study demonstrated a transient rise of DHEA with dosing of 250 mg/kg/day and a longer duration of elevated DHEA with dosing of 1000 mg without evidence of hepatic peroxisome proliferation, though an increase in smooth endoplasmic reticulum was observed consistent with enzyme induction. Thus an enzyme induction model is more consistent with the observed data and would form the basis for an epigenetic mechanism underlying the observed hepatic tumors. Leydig cell tumors are infrequently observed in humans though in rodents are commonly caused by xenobiotics that interfere with testosterone or other steroid synthesis, increase GnRH release or GnRH receptor activation, or otherwise increase the circulating levels of lutenizing hormone (LH). The Sponsor did demonstrate evidence of a significant decrease in circulating testosterone levels and a potential for an increased LH plasma concentration in a 4-week repeated-dose oral study though the increases in circulating LH did not demonstrate dose-dependence and was seen only at some time-points and was highly variable within the treatment groups. Should this be a mechanism, however, there is data from human exposure to increased circulating LH levels that suggest the human is less sensitive to elevation of this parameter than rodents and would not be expected to develop these tumors.
- Despite plausible non-genotoxic mechanisms proposed which help assuage concerns for human risk, TAK-375 and/or metabolites have been demonstrated to have a direct genotoxic effect in an *in vitro* chromosomal aberration assay and a positive, though slight, genotoxic effect in an *in vivo/in vitro* Unscheduled DNA Synthesis assay conducted in rat liver.

Study no.: M-11-00561.002A

Volume #, and page #: N/A

Conducting laboratory and location: L

Date of study initiation: October 19, 1999

GLP compliance: Yes (JAPAN only)

QA report: yes (X) no ()

Drug, lot #, and % purity: TAK-375, Lot # M375-014, 015, 016, 018, 019, 020, 021, 022; purity L J respectively

CAC concurrence: No. See Appendix I.**Methods**

Doses: 0 (vehicle – 2 groups; 0.5 w/v% methylcellulose), 15, 60, 250 or 1000 mg/kg/day TAK-375.

Test group	Dose Level (mg/kg/day)	Concentration of test suspension (mg/mL)	Dose volume (mL/kg)	Sex	Main Group		Satellite Group	
					No. of animals	Animal number	No. of animals	Animal number
Control-I	0	0	5	Male	60	1001-1060	—	—
				Female	60	1101-1160	—	—
Control-II	0	0	5	Male	60	2001-2060	—	—
				Female	60	2101-2160	—	—
Low	15	3	5	Male	60	3001-3060	10	3201-3210
				Female	60	3101-3160	10	3301-3310
Middle	60	12	5	Male	60	4001-4060	10	4201-4210
				Female	60	4101-4160	10	4301-4310
High	250	50	5	Male	60	5001-5060	10	5201-5210
				Female	60	5101-5160	10	5301-5310
Highest	1000	200	5	Male	60	6001-6060	10	6201-6210
				Female	60	6101-6160	10	6301-6310

Basis of dose selection: Doses were selected based on an estimated MTD from previous findings from several toxicokinetic studies in rats which were not designed to select a dose for a 2-year bioassay. These studies included a 26-week, 4-week and 2 week repeat dose study in Sprague-Dawley rats. A 13-week dose range-finding toxicokinetic study in the Wistar Han strain of rat and a 4-week study in this same strain were not described as being used for dose selection in this study report, however it appears these studies were initially used in the justification for dosing as submitted for Executive CAC concurrence as noted in meeting minutes. Nevertheless, the Sponsor noted that the 26-week repeat-dose toxicokinetic study in the Sprague-Dawley rat with dosing up to 150 mg/kg/day demonstrated evidence of hepatic enzyme induction with dosing $\geq$ 40 mg/kg/day but did not define an MTD. A 4-week study with oral gavage dosing up to 600 mg/kg/day was associated with a 7% suppression of bodyweight gain in males in the 600 mg/kg/day dose level while a 2-week oral gavage study of TAK-375 with dosing up to 2000 mg/kg/day revealed mortality and excessive morbidity at 2000 mg/kg/day in both males and females. From these studies, the selected high dose for the 2-year bioassay was 1000 mg/kg/day. Other doses were selected to represent an approximate common ratio of 4 from doses below and above.

Species/Strain: CD(SD)IGS SPF rats

Number/sex/group (main study): 60 animals/sex/group

Route, formulation, volume: Oral gavage, solution, 5 mL/kg

Frequency of dosing: Once daily for 104 weeks, except for females in 1000 mg/kg/day group which were terminated after 93 weeks of dosing.

Satellite groups used for toxicokinetics or special groups: 10 animals/sex/group for low, middle, high and highest dose level.

Age: 6 weeks at initiation of study

Animal housing: Individually with a large cage separated into two halves by a divider.

Restriction paradigm for dietary restriction studies: Not applicable. Pellet diet

() and drinking water were available *ad libitum*.

Drug stability/homogeneity: Drug stability of lots used in the present study was determined by analysis of a separate lot (Lot # M375-006) that was considered equivalent in quality and performance in accelerated stability tests though its manufacturing process was different. Additionally, Lot # M375-021 and 022 were not used in the study and all others were used within 36 months after the first certificate of analysis for each lot.

Dual controls employed: Yes

Interim sacrifices: Not done

Statistical analysis: See Sponsors description reproduced below:

Statistical analyses were done according to the following procedure. Data from 2 identical control groups were totalized and treated as one control group.

1) Survival Rate

After estimation of the survival curves by Kaplan-Meier's¹¹ method, the trend of survival rate to dose level was analyzed by Tarone-type method¹², and then the difference between the control group and each dose group was compared using log-rank test¹³. The lowest level of significance was 5% in two-tailed level.

2) Body Weight, Food Consumption and Hematological Data

First, the data were tested by Bartlett's test^{4,5)} for homogeneity of variance. When the variances were homogeneous, Williams' test⁶⁾, which assumes a dose-related trend, was performed to determine the non-toxic dose level. If there were no significant differences with Williams' test, Dunnett's test⁷⁾ was performed to compare the mean in the control group with that in each dose group. When the variances were heterogeneous, the Shirley-Williams test⁸⁾, which assumes a dose-related trend, was performed to determine the non-toxic dose level. If there were no significant differences with the Shirley-Williams test, the Steel test^{9,10)} was performed to compare the mean rank in the control group with that in each dose group. Bartlett's test was conducted at the two-tailed significance level of 0.05, the Williams' and Shirley-Williams tests were conducted at the two-tailed significance level of 0.05, and the other tests were conducted at the two-tailed significance levels of 0.05 and 0.01.

3) Tumor Incidence

Tumors that occurred with a frequency of more than 5% in either the control or any dose group were assessed by Peto's method¹¹⁾ to assess dose-dependency for all groups and to compare the incidence between the control group and each dose group (level of significance: 5% one-tailed level). In addition, Fisher's exact test¹²⁾ was applied to assess the difference of tumor incidence between the control group and each dose group (level of significance: 5% one-tailed level). As for hepatocellular tumors and Leydig tumor, Peto's method and Fisher's exact test were also applied to compare the incidence between each control group (control-I and control-II) and each dose group.

Deviations from original study protocol: 1) Brief deviations from temperature and humidity limits lasting less than 2 hr. 2) Brief deviation from limits described for refrigeration of dose preparations lasting 1 hr or less (9°C vs. 8°C upper limit). 3) Slight deviation from stated body weight range limits at initiation of study. 4) Lost samples for histopathological examination due to autolysis or inadequate sampling in several tissues/organs occasionally occurred and are described in the table. Nevertheless, adequate numbers of tissues were available from each group for analysis according to the Sponsor.

Observation Times

Mortality and clinical signs: All animals were observed once a day during the acclimatization period while during the study period all animals were observed three times daily: prior to dosing, immediately after dosing and 1 hr after dosing except on Saturdays and holidays in which animals were not observed 1 hr post-dose. Animal were examined for clinical signs including external appearance, nutritional condition, posture, behavior and excretions. Palpation was done once a week to detect superficial tumors. Moribund animals were necropsied after collection of blood samples and if possible, animals found dead were necropsied as well.

Body weights: Animals weighed 4 times during acclimatization period, then twice a week during the first week (Day 1 and 7) then once weekly thereafter at 7 day intervals.

Food consumption: Food consumption was measured twice during the first week (Day 1 and Day 7) and thereafter once weekly every 7 days with daily consumption determined by taking the 6 day food consumption total and determining the average.

Hematology: Blood samples were taken from the abdominal aorta under anesthesia and in a non-fasting condition from all survivors at the termination of the study. The following hematologic parameters were analyzed: red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte ratio, platelet count, white blood cell count (WBC), differential leukocyte count. Sponsor notes that "When neoplastic lesions of the blood cells were suspected from the hematologic and/or pathologic findings such as anemia or enlargement of the lymph nodes, liver or spleen, examination was done as an aid to histopathological diagnosis".

Gross pathology: All organs/tissues in the cephalic, thoracic and abdominal cavity were examined macroscopically for gross lesions. Animals found dead were examined for gross lesions if possible.

Histopathology: Peer review: yes (X), no ()

Peer reviewing pathologist:

1

The following organs/tissues were assessed microscopically: cerebral cortex, cerebellum, spinal cord (cervical, thoracic, lumbar), sciatic nerve**, eyes*, Harderian glands*, pituitary, thyroids (including parathyroids)*, adrenals*, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, thoracic aorta, trachea, lungs (including bronchus), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, submandibular glands*, sublingual glands*, liver, gallbladder, pancreas, kidneys*, urinary bladder, testes*, epididymides*, prostate, seminal vesicles*, ovaries*, uterus (both horns), vagina, mammary gland (females)*, sternum (including bone marrow), femur (including bone marrow)**, femoral muscle (skeletal muscle)**, skin (inguinal)**, and gross lesions. Additionally, Zymbal's glands (both sides), oviducts (both sides), nasal cavity and larynx tissue were preserved. Single asterisk indicates that the pair of organs were examined while a double asterisk indicates that the pair of organs/tissues were removed but one side was examined while the other was preserved for examination if necessary.

Toxicokinetics: Satellite rats from TAK-375 treated groups were subjected to blood collection from the cervical vein on three study days during the course of the 2-year bioassay: Day 1 (1st dose), Week 26 (178th dose ♂; 177th dose ♀) and Week 52 (359th dose ♂; 358th dose ♀). Three (3) animals/sex/time-point were used and 7 time-points were obtained for all but the Day 1 assessment. Time-points included were pre-dosing (except Day 1), 0.25, 0.5, 1, 3, 6 and 24 hr. Animals were unanesthetized and non-fasting at the time of blood collection. Samples were sent for testing by Sponsor using $\lceil$ method for concentration determination of test article and metabolites M-I, M-II, M-III and M-IV in each plasma sample. C_{max} and T_{max} could be determined directly while AUC_{0-24hr} was calculated based on the trapezoidal method.

Results

Mortality:

A significant reduction in survival compared with control was only observed in females dosed at the highest dose (i.e. 1000 mg/kg/day) of TAK-375 which required early termination during week 94. At the time of early termination, the survival rate for 1000 mg/kg/day females was 40.0% versus 55.0% with combined controls, $p < 0.05$). The Sponsor's table reproduced below appears to show little difference between high dose and control females but this is due to the different durations of drug administration for these groups (i.e. 104 weeks vs. 93 weeks, respectively). TAK-375 administered at doses used in this study was not associated with increased mortality in male rats, however. In fact a statistically significant increase in rate of survival was observed in the males administered 250 mg/kg/day TAK-375.

Cumulative Deaths per Treatment Group and Resulting Survival Rate

Sex	Male					Female				
	0	15	60	250	1000	0	15	60	250	1000 ^{a)}
Dose (mg/kg/day)										
No. of animals used	120	60	60	60	60	120	60	60	60	60
Week of administration										
26	1 ^{b)}	0	1	1	5	2	1	0	0	5
52	7	0	4	5	8	5	1	4	0	11
78	27	14	10	11	13	26	12	12	8	25
104	84	37	34	26	35	75	43	35	32	36 ^{d)}
Survival rate (%)	30.0	38.3	43.3	56.7 [*]	41.7	37.5	28.3	41.7	46.7	40.0 ^{e)}

a): Necropsied after week 93 of administration (in week 94 of administration)

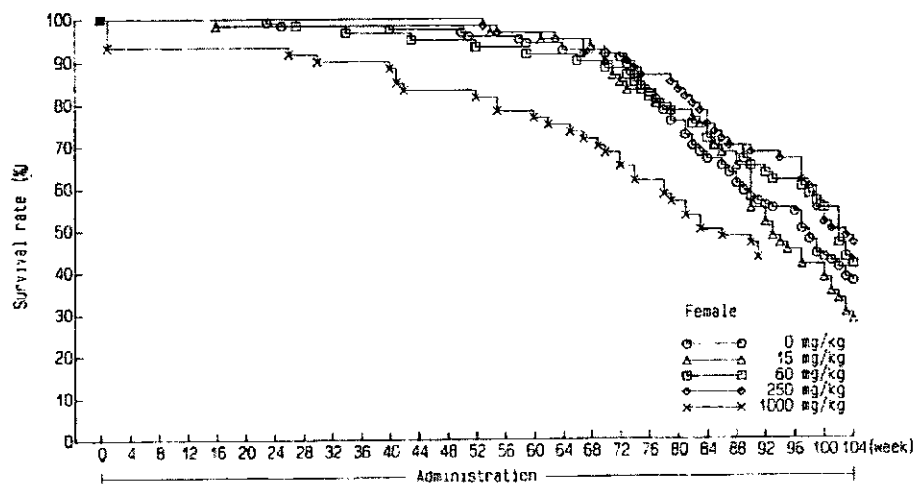
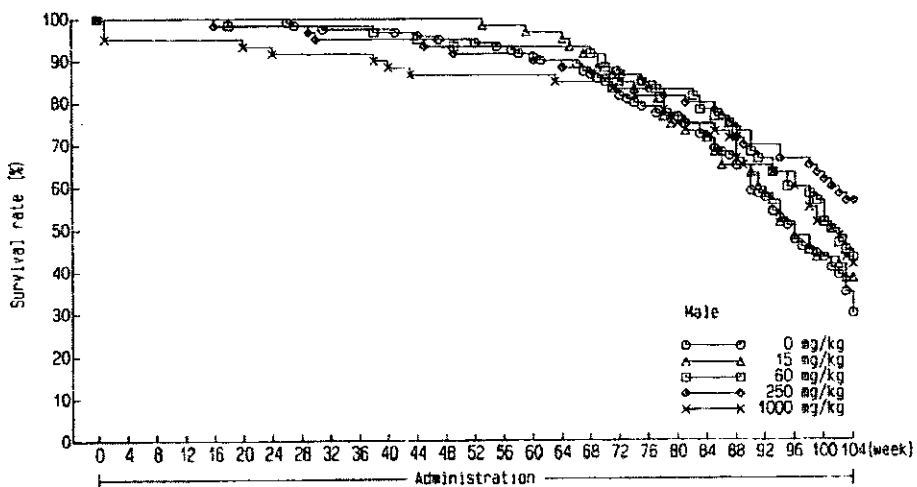
b): Cumulative number of deaths

c): Including 2 females (Nos. 6101, 6105) that died or were sacrificed moribund during the necropsy period (week 94 of administration)

d): Comparison with the control mean value in week 93

e): $p < 0.05$ (significantly different from the control group)

Below are the Sponsor's figures for rat survival during the course of the 2-year bioassay in males (top) and females (bottom).



Based on the analysis of clinical findings, gross and histopathologic assessment a presumptive cause of death was assigned to each animal that died prior to the termination of the study. The Sponsor's table characterizing these causes is reproduced below.

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On Original

Sex	Male					Female				
	0	15	60	250	1000	0	15	60	250	1000
Dose (mg/kg/day)										
No. of animals used	120	60	60	60	60	120	60	60	60	60
No. of death	84	37	34	26	35	76 ^{a)}	43	35	32	36
Test substance toxicity										
unspecified	0	0	0	0	7	0	0	0	0	6
hepatic	0	0	0	0	17	0	0	0	0	14
Tumor										
Adrenocortical tumor	0	0	0	0	0	1	0	0	0	0
Adrenomedullary tumor	1	1	0	0	0	0	0	1	0	0
Brain tumor	0	0	0	1	0	0	1	0	1	0
Bone tumor	0	0	0	1	0	0	0	0	0	0
Choriocarcinoma	0	0	0	0	0	0	0	1	0	0
Heart tumor	1	0	0	0	0	0	0	0	0	0
Histiocytic tumor	2	0	1	0	2	0	0	0	1	0
Leukemia, lymphoid	0	0	0	0	0	0	0	1	0	0
Leukemia, myeloid	1	1	0	1	0	0	0	0	1	0
Mammary tumor	0	0	0	0	0	9	5	6	5	0
Malignant lymphoma	1	0	0	0	0	3	0	0	0	0
Oral tumor	1	0	0	0	0	0	0	0	0	0
Pituitary tumor	32	10	18	12	6	56	33	22	21	7
Zymbal gland tumor	0	1	0	0	0	0	0	0	0	0
Prostate tumor	1	1	0	0	0	-	-	-	-	-
Renal tumor	2	0	0	0	0	0	0	1	0	0
Splenic tumor	0	0	1	0	0	0	0	0	0	0
Subcutaneous tumor	6	5	1	0	0	2	0	0	0	0
Uterine tumor	-	-	-	-	-	1	0	0	1	0
Vaginal tumor	-	-	-	-	-	1	0	1	0	0
Non-tumor										
Tooth abnormalities	0	2	0	1	0	1	0	0	1	0
Brain lesion	1	0	0	0	0	0	0	0	0	0
Cardiovascular lesion	1	0	0	0	0	0	0	0	0	0
Foot lesion	2	0	0	0	0	0	0	0	0	0
Liver lesion	1	0	0	0	0	0	0	0	0	0
Renal lesion	7	6	0	1	0	1	0	0	0	1
Lung lesion	1	0	0	0	0	0	0	1	0	0
Sepsis	0	1	0	0	0	0	0	0	0	0
Spinal cord lesion	0	1	0	0	0	0	0	0	0	0
Urogenital tract lesion	2	0	1	1	0	0	0	0	0	0
Intubation error	1	0	0	1	0	0	1	0	0	6
Unclear	20	8	12	7	3	1	3	1	1	2

a): Including one male (No. 1109) that died during necropsy period

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According to the Sponsor's analysis, the majority of premature deaths with dosing ≤ 250 mg/kg/day in males and females were associated with development of pituitary tumor while those occurring at doses of 1000 mg/kg/day were primarily caused by "test-article toxicity" which was considered unspecified if the animal died shortly after dosing and displayed clinical signs of lateral or prone position which was interpreted as evidence of TAK-375 intoxication. Little gross or hisopathologic evidence of organ damage or tissue alterations were found in these animals. Hepatic toxicity was considered the cause of death in 17 males and 14 females between weeks 71 and 103 (males) or 65 – 94 (females). Upon gross and microscopic examination, marked liver alterations and tumors were observed which were thought to have produced an altered hepatic status or dysfunction.

Clinical signs:

Clinical signs which appeared to be related to TAK-375 administration and were dose-dependent were limited to increased incidence of abnormal gait, prone/lateral position following dosing, oligpnea (shallow and shallow breathing), distension of abdomen and clonic convulsion or other convulsion. Abnormal gait, altered position and oligopnea were generally observed during the first week of administration but disappeared by Week 2 with sporadic observation in males after Week 23 and in females after Week 4. Typically this increased incidence was not observed with dosing ≤ 250 mg/kg/day but was observed only with the 1000

mg/kg/day dose level when compared with control. The exception to this was found in females in which administration of TAK-375 appeared to dose-dependently increase the frequency of convulsions with dosing ≥ 60 mg/kg/day though the frequency was low in all groups with the exception of the high dose. See table below for details.

TAK-375 Dose (mg/kg/day)										
Dose	Males					Females				
	0	15	60	250	1000	0	15	60	250	1000 [†]
n	120	60	60	60	60	120	60	60	60	60
Clinical Sign										
Abnormal gait	0 0%	0 0%	0 0%	0 0%	5 8%	0 0%	0 0%	0 0%	0 0%	7 12%
Prone/Lateral position	12 10%	3 5%	11 18%	8 13%	22 37%	19 36%	10 17%	7 12%	6 10%	24 40%
Oligopnea	31 26%	11 18%	14 23%	13 22%	29 48%	41 34%	20 33%	23 39%	13 22%	31 52%
Distension of abdomen	0 0%	1 2%	0 0%	0 0%	4 7%	1 1%	0 0%	2 3%	0 0%	7 12%
Clonic convulsion/ Convulsion	5 4%	3 5%	3 5%	3 5%	12 20%	1 1%	0 0%	2 3%	3 5%	8 13%

[†], females sacrificed during Week 94 (93-weeks of dosing)

The Sponsor's table listing all the observed clinical signs is reproduced below:

Incidence of Observed Clinical Signs by Treatment Group

Sex	Males					Females				
	0	15	60	250	1000	0	15	60	250	1000 ^{a)}
Dose (mg/kg/day)	0	15	60	250	1000	0	15	60	250	1000 ^{a)}
No. of animals used	120	60	60	60	60	120	60	60	60	60
Decrease, spontaneous movement	25	9	8	11	14	42	19	25	18	20
Abnormal gait (staggering gait)	0	0	0	0	5	0	0	0	0	7
Prone/lateral position	12	3	11	8	22	19	10	7	6	24
Hypothermia	5	1	4	4	6	8	4	8	5	9
Oligopnea	31	11	14	13	29	41	20	23	13	31
Emaciation	7	7	4	2	2	15	3	3	5	1
Distension, abdomen	0	1	0	0	4	1	0	2	0	7
Torticollis	0	1	1	0	0	5	1	6	2	1
Clonic convulsion/convulsion	5	3	3	3	12	1	0	2	3	8

a): Necropsied after week 93 of administration (in week 94 of administration)

The incidences of palpable masses detected are reproduced below from the Sponsor's table.

Incidence of Palpable Masses by Treatment Group

Sex	Males					Females				
	0	15	60	250	1000	0	15	60	250	1000 ^{a)}
Dose (mg/kg/day)	0	15	60	250	1000	0	15	60	250	1000 ^{a)}
No. of animals used	120	60	60	60	60	120	60	60	60	60
No. of palpable mass-bearers	56	28	20	20	8	72	31	34	21	2

a): Necropsied after week 93 of administration (in week 94 of administration)

No dose-related increase in palpable masses was observed for TAK-375-treated animals relative to controls. Interestingly, the number of palpable mass-bearers was greatly lower in the 1000 mg/kg/day group compared with all others (Percentage with palpable masses at 0, 15, 60, 250 and 1000 mg/kg/day were: males = 47%, 47%, 33%, 33% and 13%; females = 60%, 52%, 57%, 35% and 3%, respectively), an observation which cannot be explained by early

termination of high dose animals as this was only done in females while males at the high dose were terminated at the end of the study along with all other groups. Statistical analysis was not conducted on these data, however.

Body weights:

Male and female rats administered 15 or 60 mg/kg/day of TAK-375 did not demonstrate a significant reduction in body weight from controls at the termination of the study nor was there a significant reduction in body weight gain during the course of the study. Transient statistically significant reductions in body weight compared to control were observed starting during the early portion of the study but which were not maintained as the study progressed. Male rats at 15 mg/kg/day had a reduced body weight during weeks 9 – 24, males at 60 mg/kg/day from weeks 2 – 63. Females at 15 and 60 mg/kg/day demonstrated a reduced body weight compared with controls between weeks 4 – 35. End of study effects on body weight and body weight gain were observed with male and female dose administration of ≥ 250 mg/kg/day as shown in Sponsor’s table below.

Body Weight and Body Weight Gain by Treatment Group

Sex	Males				Females			
	15	60	250	1000	15	60	250	1000 ^{a)}
Dose (mg/kg/day)	15	60	250	1000	15	60	250	1000 ^{a)}
No. of animals used	60	60	60	60	60	60	60	60
Mean body weight at the end of administration	N	N	-10%*	-39%*	N	N	-19%*	-44% ^{a)}
Body weight gain (0 - 104 w)	N	N	N	-53%*	N	N	-29%*	NA

Values in the table indicate change of percentage against the mean control value

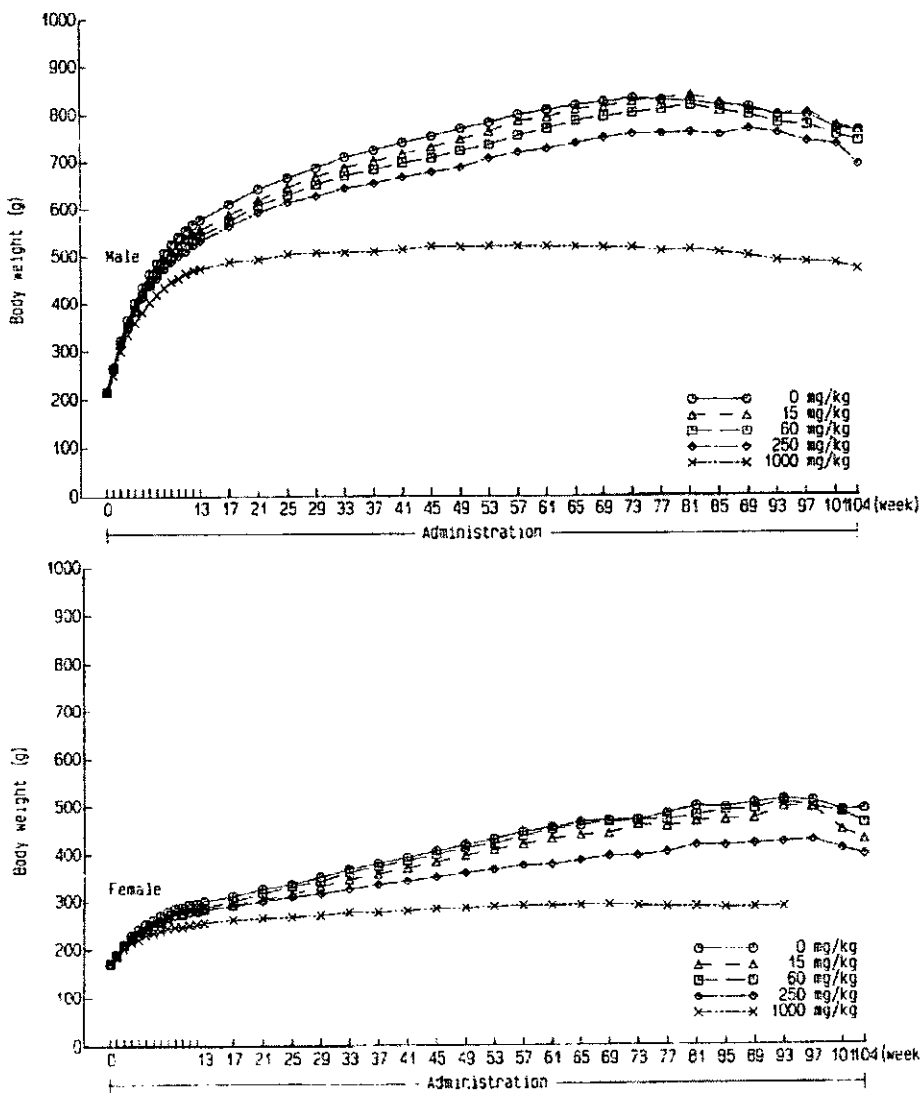
N: No remarkable change, -: Decrease, NA: not available

a): Necropsied after week 93 of administration (in week 94 of administration)

b): Comparison with the control mean value at week 93

*: $p < 0.05$ (significantly different from the control group)

In males, a dose of 250 mg/kg/day produced a reduction in body weight of 10% compared with controls while which was increased to 39% at a dose of 1000 mg/kg/day. Additionally, body weight gain, which did not appear different from control at the 250 mg/kg/day level was 53% lower in the 1000 mg/kg/day than their respective control. Female rats administered 250 mg/kg/day had body weight reductions of 19% compared with control animals and when assessing mean body weight gain these animals demonstrated a 29% reduction in weight gain during the study compared with controls. The 1000 mg/kg/day TAK-375 dose was associated with tremendous weight loss ($\downarrow 44\%$) after 93 weeks when compared with controls at the same point in the study. Below is the Sponsor’s figure depicting body weight in males (top) and females (bottom) as a function of week on study. In both males and females of the 1000 mg/kg/day dose group, body weight appears to reach a plateau by Week 17 while in males in lower dose groups and control group weight increased through roughly the 85th week while in females weight increased in other groups through the 97th week.



Food consumption:

No significant differences were found between control and TAK-375 treated groups.

Hematology:

Limited alteration of hematologic parameters was observed with dosing ≤ 250 mg/kg/day. Small but statistically significant changes in mean cell volume and mean cell hemoglobin were observed in females but other parameters were unchanged relative to controls in both males and females. Evidence of TAK-375-induced effect on hematology was observed primarily at the 1000 mg/kg/day dose level in both male and female rats. Males administered 1000 mg/kg/day for 104 weeks exhibited an elevation in RBC and mean cell hemoglobin concentration values compared with control animals while mean cell volume and mean cell hemoglobin were reduced. Females appeared to demonstrate a broad and significant reduction in hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin and reticulocyte ratio while mean cell hemoglobin concentration – as in males – increased slightly but significantly.

In both males and females, a significant thrombocytosis was observed and in females an increase in white blood cell number. Leukocyte differential was not conducted to further characterize the nature of the WBC increase.

Text Table 6. Summary of major hematological data

Sex	Males				Females			
	15	60	250	1000	15	60	250	1000 ^{a)}
Dose (mg/kg/day)								
No. of animals used	23	26	34	25	17	25	28	24
RBC	N	N	N	+14%*	N	N	N	N
Hb	N	N	N	N	N	N	N	-8%*
Ht	N	N	N	N	N	N	N	-13%*
MCV	N	N	N	-10%*	N	N	-5%*	-12%*
MCH	N	N	N	-5%*	N	N	-5%*	-8%*
MCHC	N	N	N	+6%*	N	N	N	+4%*
Reticulocyte	N	N	N	N	N	N	N	-11%*
Platelet	N	N	N	+29%*	N	N	N	+59%*
WBC	N	N	N	N	N	N	N	+37%*

Values in the table indicate change of percentage against the mean control value

N: No remarkable change, +: Increase, -: Decrease

a): Necropsied after week 93 of administration (in week 94 of administration)

*: p<0.05 (significantly different from the control group)

Gross pathology:

Macroscopic examination of tissues collected upon sacrifice of rats used in the study revealed several apparent TAK-375-related alterations which exhibited a dose-response relationship. Generally these related to dark discoloration of several organs/glands in male and female rats including the kidney and extraorbital lacrimal gland (observed primarily in females), as well as the liver and thyroid gland (males and females). Focal discoloration of the lung appeared to be slightly elevated in high dose male and female animals while testicular discoloration was clearly dose-dependent. Other gross reproductive observations included an increased frequency of small prostate and seminal vesicle with increasing TAK-375 dose. See the table reproduced below.

Macroscopic Observations by Treatment Group

Group	Males						Females					
	C1	C2	LD	Low MD	High MD	HD	C1	C2	LD	Low MD	High MD	HD [†]
Dose (mg/kg/day)	0	0	15	60	250	1000	0	0	15	60	250	1000
N	60	60	60	60	60	60	60	60	60	60	60	60
Adrenal												
Discoloration, yellowish-white	0	0	0	0	0	7	0	0	0	0	0	0
Large	5	4	6	2	5	15	10	11	7	13	20	11
Extraorbital lacrimal gland												
Discoloration, dark	0	0	0	0	0	4	0	0	0	0	12	32
Kidney												
Discoloration, dark	0	0	1	0	0	6	0	0	0	0	0	2
Liver												

Discoloration, dark	0	0	0	0	1	42	0	0	0	0	12	49
Large	0	0	1	0	6	50	0	1	1	1	12	53
Node	0	3	6	2	10	46	1	3	0	7	19	49
Focus, raised	0	1	0	3	0	0	0	1	1	2	4	7
Focus, discolored	12	9	11	20	29	46	9	18	17	22	33	36
Lung (bronchus)												
Focus, discolored	2	2	5	4	8	17	2	1	3	5	5	13
Prostate												
Small	1	1	1	2	3	10						
Seminal vesicle												
Small	2	2	1	2	4	17						
Testis												
Focus, discolored	2	0	1	0	7	46						
Thyroid												
Discoloration, dark	0	0	0	0	26	53	0	0	0	0	9	53

Histopathology:

Neoplastic:

The table reproduced from Sponsor's submission below documents all neoplastic lesions which were observed in any treated group at an incidence of >5%. Selected TAK-375 related neoplastic lesions are extracted in a subsequent table.

Incidence of Neoplastic Lesions by Treatment Group

Sex	Male						Female					
	0		15	60	250	1000	0		15	60	250	1000
Dose (mg/kg/day)	60	60	60	60	60	60	60	60	60	60	60	60
No. of animals used	60	60	60	60	60	60	60	60	60	60	60	60
Adrenal	(n=59) ^{a)}		(n=59)									
Adenoma, cortical cell	2	0	1	2	0	2	0	4	1	1	4	2
Carcinoma, cortical cell	0	0	0	0	0	0	1	0	1	0	0	0
Pheochromocytoma	7	4	9	6	2	1*	2	4	0	3	1	1
Pheochromocytoma, malignant	0	0	1	0	1	0	2	0	0	1	0	0
Hemolymphoreticular (all sites)												
Sarcoma, histiocytic	2	1	0	2	1	3	0	1	0	0	2	1

Liver												
Adenoma, hepatocellular	0 [‡]	3	7** [†]	7** [†]	15** [†]	47** [†]	1 [‡]	1	0	8** [†]	22** [†]	49** [†]
	0.0000 [‡]		0.0085 [†]	0.0132	0.0000	0.0000	0.0000			0.0020	0.0000	0.0000
Carcinoma, hepatocellular	0 [‡]	0	0	0	0	38** [†]	0 [‡]	0	0	1	1	20** [†]
	0.0000					0.0000	0.0000					0.0000
Hepatoblastoma	0	0	0	0	0	1	0	0	0	0	0	0
Mammary gland												
Adenoma	1	0	0	0	0	0	1	1	4 [‡]	3	3	0
Adenocarcinoma	0	0	0	0	0	0	13	16	14	17	11	0*
Fibroadenoma	1	0	2	0	0	0	32	24	21	25	20	4*
Pancreas												
Adenoma, acinar cell	2	4	7	7	7	2	1	1	0	2	3	0
Adenoma, islet cell	23	24	16	23	28	15*	6	4	8	6	4	0*
Carcinoma, islet cell	1	2	0	0	1	0	0	1	0	0	0	0
Pituitary												
Adenoma, anterior	(n=59) [†]		(n=59)		(n=59)	(n=59)			(n=59)		(n=58)	
	40	44	42	42	40	22*	49	48	48	44	45	22*
Carcinoma, anterior	0	0	0	1	1	0	2	3	4	1	3	0
Skin												
Fibroma	6	1	3	3	2	1	0	0	0	0	0	0
Fibrosarcoma	2	2	2	0	2	1	1	0	0	0	0	0
Keratoacanthoma	1	0	4** [†]	2	4** [†]	0	0	1	0	1	0	0
Papilloma, squamous cell	0	2	5** [†]	2	4** [†]	1	0	0	0	0	0	0
Testis												
Leydig cell tumor	3 [‡]	0	1	0	7** [†]	46** [†]	-	-	-	-	-	-
	0.0000				0.0175	0.0000						
Thyroid												
Adenoma, C cell	(n=41)	(n=46)	(n=49)	(n=47)	(n=51)	(n=53)	(n=53)	(n=57)	(n=53)	(n=56)	(n=47)	(n=53)
	6	2	2	4	2	3	2	3	7 [†]	5	1	0
Carcinoma, C cell	0	1	0	0	1	1	0	3	0	2	1	0
Adenoma, follicular cell	0	1	2	0	4 [‡]	1	0	0	0	1	0	0
Carcinoma, follicular cell	0	0	0	1	0	1	0	0	1	0	0	0
Uterus												
Granular cell tumor	-	-	-	-	-	-	1 [‡]	2	1	1	3	1
Polyp, endometrial stromal	-	-	-	-	-	-	5	3	5	2	7	2
Vagina												
Granular cell tumor	-	-	-	-	-	-	4	2	2	1	5	0

a): control-I

b): control-II

c): Peto's positive trend test p- value of pooled tumor incidence for all groups

d): Pairwise comparison of pooled tumor between the dosage group and the control (I+II) group

e): Effective number

^{*}: p<0.05 (significantly different from the control [total of the control I & II] group, Fisher's exact test)

[†]: p<0.05 (significantly different from the control [total of the control I & II] group, Peto's test)

[‡]: p<0.05 (significantly different from the control [total of the control I & II] group, Peto's positive trend test)

[‡]: No significant difference from the control-II in Fisher's exact and Peto's tests

[‡]: No significant difference from the control-I in Fisher's exact and Peto's tests

TAK-375-associated tumors which were observed to increase in incidence or severity with increasing dose are in the following table which is adapted from the Sponsor's previous table. Note that the trend test and pairwise statistical analysis conducted by Dr. Joan Buenconsejo

utilized different levels of significance for common tumors ($p < 0.005$ and $p < 0.01$, respectively) defined as being observed in $\geq 1\%$ of control animals versus rare tumors ($p < 0.025$ and $p < 0.05$, respectively) which were below 1% incidence in controls.

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Apparent TAK-375-Related Neoplasms in 104-Week Rat Bioassay (Combined Early and Terminal Sacrifice)

Group	Males						Females					
	C1	C2	LD	Low MD	High MD	HD	C1	C2	LD	Low MD	High MD	HD [†]
Dose (mg/kg/day)	0	0	15	60	250	1000	0	0	15	60	250	1000
N	60	60	60	60	60	60	60	60	60	60	60	60
Liver												
<i>Adenoma, hepatocellular</i>												
Incidence	0 [§]	0	7	7	15 [‡]	47 [‡]	1 [§]	1	0	8 [‡]	22 [‡]	49 [‡]
%	0%	0%	12%	12%	25%	78%	2%	2%	0%	13%	37%	82%
Historical Control (Mean and Max) [#]	3.8% (max: 8.3%)						1.3% (max: 1.7%)					
Historical Control (Mean and Max) [@]	2.0% (max 8.0%)						1.6% (max 13.3%)					
<i>Carcinoma, hepatocellular</i>												
Incidence	0 [§]	0	0	0	0	38 [‡]	0 [§]	0	0	1	1	20 [‡]
%	0%	0%	0%	0%	0%	63%	0%	0%	0%	2%	2%	33%
Historical Control (Mean and Max) [#]	0.8% (max: 1.7%)						0% (max: 0%)					
Historical Control (Mean and Max) [@]	2.2% (max 6.7%)						0.3% (max 1.7%)					
Testis												
<i>Tumor, Leydig Cell</i>												
Incidence	3 [§]	0	1	0	7	46 [‡]						
%	5%	0%	2%	0%	12%	77%						
Historical Control (Mean and Max) [#]	1.7% (max: 2.5%)						N/A					
Historical Control (Mean and Max) [@]	2.4% (max 9.3%)											

[†], females at 1000 mg/kg/day were sacrificed during the 94th week of administration
[#], From Takeda; () database of spontaneous neoplasms in control — :CD(SD)IGS rats in 104-week bioassays (3 studies, 240 animals/sex)
[@], From C () spontaneous neoplasms in control — CD@ (SD) rats in 104-week bioassays (31 studies; 12 — colony-derived); ()
[§], trend is significant (Peto's positive trend test)
[‡], significantly different from combined controls (p<0.01)

Note: For microscopic observations of neoplastic lesions in all animals, those with unscheduled death and those terminated at end of study please see Appendices 3 – 5.

Three tumors appeared to be directly associated with TAK-375 exposure in the rat bioassay: adenoma and carcinoma of the liver of both male and female rats and Leydig cell tumor in the male rat. Several tumor types, such as pituitary adenoma, mammary gland adenoma and fibroadenoma and pancreatic adenoma and potentially adrenal pheochromocytoma were observed to decrease with increasing TAK-375 dose, a finding which is thought by the Sponsor, and with which this reviewer agrees, likely to be secondary to changes in weight and potentially the early termination of the high dose females. The frequency of parathyroid adenoma was statistically increased in male rats with increased dosing though but was ignored by the Sponsor evidently due to the low incidence (i.e. < 5% in any dose level) and/or the lack of this finding in the female rats. In addition, the incidence of parathyroid adenoma is well within the range of historical control data obtained for this strain of rat.

The table submitted by the Sponsor below illustrates the increase in benign and malignant tumors observed in treated animals relative to controls. Clear increases in both benign and malignant tumors are observed in male rats while in female rats a decrease in mammary gland tumors and pituitary tumors masked the increase in hepatic tumors observed with increases in TAK-375 dose.

Tumors and Tumor-bearing Rats by Treatment Group

Sex	Male						Female					
	0		15	60	250	1000	0		15	60	250	1000
Dose (mg/kg/day)	60	60	60	60	60	60	60	60	60	60	60	60
No. of animals used	60	60	60	60	60	60	60	60	60	60	60	60
No. of benign tumors	122	99	124	130	160	328	131	137	124	131	189	316
No. of malignant tumors	12	15	9	9	12	86	31	36	27	35	35	37
No. of tumors	134	114	133	139	172	414	162	173	151	166	224	353
No. of benign tumor bearers	52	51	50	50	52	52	55	51	56	50	56	50
No. of malignant tumor bearers	11	14	9	8	12	40	24	22	25	24	19	22
No. of multiple tumor bearers	35	31	38	40	41	52	45	45	46	40	43	46
No. of tumor bearing animals	54	55	51	51	54	52	60	57	58	57	57	52

Non-neoplastic: The following histopathologic observations were noted to occur more frequently in TAK-375-treated mice compared with their respective control groups. Note that no statistical analysis was conducted by Sponsor.

Histopathologic (Non-neoplastic) Findings by Treatment Group

Group	Males						Females					
	C1	C2	LD	Low MD	High MD	HD	C1	C2	LD	Low MD	High MD	HD [†]
Dose (mg/kg/day)	0	0	15	60	250	1000	0	0	15	60	250	1000
N	60	60	60	60	60	60	60	60	60	60	60	60
Adrenal												
Vacuolation, cortical cell	6	11	9	18	41	52	1	2	1	28	48	47
Vacuolation, cortical cell focal	12	9	7	6	16	46	3	4	4	3	3	14
Foamy cell, cortex	0	0	0	0	1	4	0	0	0	0	0	0
Hypertrophy, cortical cell, diffuse	0	1	1	9	35	34	4	1	3	9	48	46
Cerebral cortex												
Pigmentation, PAS negative	0	0	0	0	10	50	0	0	0	0	19	45
Mineralization	2	0	0	1	2	28	2	0	0	0	0	6
Cerebellum												
Pigmentation, PAS negative	0	0	0	0	11	50	0	0	0	0	8	46
Epididymidis												
Atrophy	19	15	21	8	9	28						
Hypospermia	18	12	18	8	8	36						
Extraorbital lacrimal gland												
Pigmentation, acinar cell, increased	1	0	1	0	1	21	3	2	2	5	23	44
Eye												
Atrophy, retinal	9	3	10	10	8	21	18	16	12	17	12	14
Femur & Marrow												
Atrophy, trabecular bone	21	19	11	19	26	43	3	3	4	5	9	17
Kidney												
Chronic progressive nephropathy	40	37	33	44	49	44	15	13	11	19	23	40
Pigmentation, tubular cell, increased	2	3	1	0	6	39	9	8	6	13	29	54
Hyperplasia, tubular, regenerative	0	0	1	2	0	4	0	1	0	0	1	5

Hyperplasia, tubular, simple	0	0	1	1	0	3	0	1	0	0	1	5
Liver												
Necrosis, focal	8	9	6	9	9	37	5	4	6	5	9	35
Pigmentation, sinusoid lining cell	0	0	0	0	0	54	0	0	0	0	3	50
Pigmentation, hepatocytic	0	0	0	0	2	47	0	0	0	0	51	55
Hemorrhage	2	0	2	1	0	19	1	2	0	1	2	13
Fibrosis	4	3	2	5	6	43	3	2	4	9	5	46
Hypertrophy, hepatocytic, central	0	0	3	37	49	50	0	0	0	14	48	36
Hyperplasia, bile duct, cystic	0	0	0	0	2	15	0	2	0	4	15	35
Proliferation, bile duct	28	21	21	32	29	41	16	22	27	22	18	11
Proliferation, oval cell	2	1	0	0	0	39	2	1	2	3	8	48
Spongiosis hepatic	17	19	25	28	39	21	3	5	3	4	14	16
Altered cell focus, amphophilic	0	0	2	5	13	32	0	0	2	3	5	21
Altered cell focus, basophilic	2	6	3	4	15	29	4	5	5	6	26	35
Altered cell focus, clear	16	21	20	32	33	39	15	17	20	26	30	25
Altered cell focus, eosinophilic	17	16	27	43	52	51	15	21	25	38	50	45
Altered cell focus, mixed	0	0	0	3	10	14	2	2	2	9	28	20
Lung												
Hemorrhage	2	0	3	6	9	12	3	4	2	8	13	12
Inflammation interstitial, focal	4	0	3	4	17	35	2	1	4	3	10	26
Histiocytosis, alveolar	28	27	28	42	52	49	31	29	30	41	52	50
Histiocytosis, alveolar, pigmented	0	2	0	5	7	32	0	1	0	3	5	25
Pituitary												
Cyst	2	1	1	2	3	6	1	1	1	0	1	3
Prostate												
Atrophy, moderate/ Severe	6	14	15	8	11	33						

Salivary gland, submandibular												
Pigmentation, macrophage	0	0	0	0	0	9	0	0	0	0	0	9
Salivary gland, sublingual												
Pigmentation, macrophage	0	0	0	0	0	3	0	0	0	0	0	4
Spinal cord												
Pigmentation, PAS-negative	0	0	0	0	8	49	0	0	0	0	12	45
Stomach												
Erosion/Ulcer, glandular stomach	11	13	5	8	7	11	9	7	6	10	9	17
Testis												
Atrophy, seminiferous tubular	19	12	17	11	18	43						
Hyperplasia, Leydig cell, focal	5	2	2	9	18	48						
Thyroid												
Cyst, ultimobranchial	3	3	3	9	3	12	4	5	6	8	12	10
Pigmentation, follicular cell	0	0	0	2	39	46	0	0	0	0	25	45
Small follicle, increased	1	5	1	5	24	45	0	3	2	7	13	37
Hyperplasia, follicular, focal	2	3	7	6	6	12	1	1	3	3	1	5

†, females at 1000 mg/kg/day were sacrificed during Week 94 of the study.

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Toxicokinetics:

Analysis of TAK-375 and metabolites M-I through M-IV on Day 1, and during Week 26 and 52 yielded the following results:

TAK-375

Analyte	Parameter	Stage	Sex Dose (mg/kg/day)	Male				Female				
				15	60	250	1000	15	60	250	1000	
TAK-375	T _{max} (h)	1st		0.4	0.3	0.3	0.8	0.3	0.3	0.7	2.7	
		26 w		0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.3	
		52 w		0.3	0.4	0.7	0.5	0.3	0.3	0.5	0.6	
	C _{max} (ng/mL)	1st			547	6059	17401	38813	1436	12806	33425	94304
		26 w			2138	6563	9838	17818	5472	10321	20245	35649
		52 w			3053	7822	12175	22333	3610	16452	23059	52831
	AUC _{0-24h} (ng·h/mL)	1st			952	10750	120809	222313	1250	29531	173941	1539651
		26 w			3178	11613	35692	90467	4924	19243	44625	146148
		52 w			4569	13432	42033	171041	4434	26478	91839	230770

TAK-375 exposure increased with dose though this was sub-dose-proportional in nature. Female mice exhibited higher exposure for the same dose of TAK-375 as compared with males; C_{max} was generally 2-fold higher in females with dosing $\geq$ 60 mg/kg/day and AUC exposure showed similar gender sensitivity. Exposure to TAK-375 was reduced with repeated-dosing at levels $\geq$ 250 mg/kg/day though this was greater at Week 26 than Week 52.

Exposure to the active metabolite M-II was similarly greater in females than in males with a greater than 2-fold difference by AUC. Exposure was generally dose-dependent and sub-proportional to dose. Reduction in exposure with repeated-dosing was observed with all dose levels $\geq$ 60 mg/kg/day in both male and female rats. As with TAK-375, the reduced exposure was of greater magnitude at the Week 26 assessment than at Week 52.

See Sponsor's table for data for M-II and the inactive metabolites M-I, M-III and M-IV which is reproduced below.

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TAK-375 Metabolites

Analyte	Parameter	Stage	Sex Dose (mg/kg/day)	Male				Female				
				15	60	250	1000	15	60	250	1000	
M-I	T _{max} (h)	1st		0.5	0.5	0.5	0.8	0.4	0.5	0.5	0.8	
		26 w		0.3	0.3	0.3	0.4	0.3	0.3	0.5	0.4	
		52 w		0.3	0.4	0.5	0.5	0.5	0.4	0.4	0.7	
	C _{max} (ng/mL)	1st		648	4457	11645	20375	1038	4708	7678	8159	
		26 w		1706	5204	7940	9354	2084	3182	3143	4730	
		52 w		1955	5301	7295	9404	1588	3573	4492	4385	
	AUC _{0-24h} (ng·h/mL)	1st		1495	9309	58271	115643	1400	8824	20699	62483	
		26 w		3406	9629	29228	45350	2845	5645	8556	25057	
		52 w		4332	9438	21755	59586	2462	6072	13434	27073	
	M-II	T _{max} (h)	1st		0.6	1.0	1.7	1.7	0.5	0.8	3.3	4.0
			26 w		0.5	0.7	0.7	0.5	0.4	0.8	0.5	0.7
			52 w		0.7	0.7	0.8	0.5	0.7	0.8	1.0	0.8
C _{max} (ng/mL)		1st		441	1869	2427	3510	767	2345	4090	5300	
		26 w		336	838	648	1050	1336	2973	3759	3332	
		52 w		427	892	804	1206	1250	3048	3671	4088	
AUC _{0-24h} (ng·h/mL)		1st		1368	8758	30131	53195	1396	17648	47156	96327	
		26 w		1476	3349	3687	7762	3555	13178	17459	24876	
		52 w		2074	2953	3359	12194	3773	9945	22278	35379	
M-III		T _{max} (h)	1st		0.7	0.8	0.8	1.7	0.5	0.8	2.3	11.0
			26 w		0.3	0.5	0.7	0.5	0.7	0.8	0.8	0.7
			52 w		0.3	0.5	1.0	2.3	0.8	0.8	1.7	0.8
	C _{max} (ng/mL)	1st		971	4416	8073	16040	1616	8449	22174	44912	
		26 w		2024	4786	6327	10187	3325	6641	11879	17208	
		52 w		2414	5780	9055	12292	2615	8530	14016	21194	
	AUC _{0-24h} (ng·h/mL)	1st		2544	14375	79244	172637	2920	56156	269058	740621	
		26 w		5568	13578	36066	73837	8262	26629	53208	102098	
		52 w		7412	14189	38089	153732	7554	25170	98970	164879	
	M-IV	T _{max} (h)	1st		1.0	1.0	3.0	4.0	1.0	1.7	6.0	24.0
			26 w		0.8	1.0	1.0	3.0	1.0	1.0	2.3	2.3
			52 w		1.0	1.0	1.7	4.0	1.7	1.7	2.3	2.3
C _{max} (ng/mL)		1st		440	911	913	1049	623	1350	1652	1422	
		26 w		244	341	245	393	686	911	980	866	
		52 w		320	356	274	482	592	741	970	1036	
AUC _{0-24h} (ng·h/mL)		1st		2146	6324	11448	18439	2172	12652	23122	30201	
		26 w		1376	1775	1770	3906	3260	6606	8328	9885	
		52 w		1968	1565	1658	6178	3685	3981	9208	13410	

Comparison of TAK-375 and M-II Metabolite Exposures at Rat Tumor NOAEL versus Expected Clinical Exposure with TAK-375 at 8 mg/day

Tumor/Type	SEX	NOAEL DOSE (mg/kg/day)	[TAK-375] (ng·h/mL)	HUMAN MOS†	[M-II] (ng·h/mL)	HUMAN MOS†
LIVER						
Adenoma	M	60	13,432	1,419-fold	2,953	12-fold
	F	15	4,434	472-fold	3,773	16-fold
Carcinoma	M	250	42,033	4,472-fold	3,359	14-fold
	F	250	91,839	9,770-fold	22,278	92-fold
TESTIS						
Leydig cell tumor	M	250	42,033	4,472-fold	3,359	14-fold
	F			N/A		
† MOS = Margin of Safety, based on results from study 01-01-TL-375-03, elderly administered 16 mg with extrapolation to 8 mg based on linear kinetics within this dose range. Estimated AUC _{0-24h} TAK-375: 9.4 ng·h/mL, M-II: 241.3 ng·h/mL.						
N.D., not determined						

Discussion and Conclusions

Summary of individual study findings:

Adequacy of carcinogenicity study and appropriateness of test model

The rat is a common animal model for the assessment of carcinogenicity in 2-year bioassays and the number of animals and duration of treatment was adequate for assessment of tumorigenicity relating to the oral administration of TAK-375. Rats were administered TAK-375 in doses which well exceed the intended dose to be given clinically through the oral route which is the intended route of administration in humans. Rats were given drug once daily as is intended in clinical use of the drug. The metabolism of TAK-375 in rats is qualitatively, though not quantitatively, similar to humans with the TAK-375 parent being much more highly represented in the plasma than in humans relative to the active metabolite M-II. TAK-375 levels in rat plasma at the lowest dose produced an exposure which was 472-fold the level of parent drug expected in humans with clinical dosing. The plasma concentration of the active metabolite M-II achieved in rats exceeded the clinical exposure at all dose levels ranging from 9 and 16-fold in male and female animals at the low dose to 51 and 147-fold at the high dose level. Thus, dose levels of TAK-375 used were sufficient to produce levels of M-II which surpass those expected to be observed in humans by nearly 10-fold and above. As such, the present study in using the clinically intended route of administration exposed this animal model to plasma concentrations of drug and active metabolite at levels which well exceed the expected human exposure for 2 years of continuous dosing.

Dosing used in this study was not previously agreed upon with the Executive Carcinogenicity Assessment Committee (ECAC) prior to initiation of the study. The common practice of conducting a 13-week dose range-finding study was not done in this strain of rat, though a dose range-finding study was conducted in a different strain, the Wistar Han rat. Dosing in the Sprague-Dawley rats used in the present study was derived from studies in both Wistar and Sprague-Dawley. Concerns during review of the carcinogenicity protocol centered on the fact

that the MTD was not defined in a long-term Sprague-Dawley study though in a previous study conducted in the Wistar rat the MTD would appear to be < 1500 mg/kg/day. From the results of the completed study, the MTD was reached and exceeded in both males and females rats apparent in the excessive body weight loss and concurrent reduction in survival which required early termination of females at this dose level. Effects observed were generally consistent from the 250 mg/kg/day dose level to the 1000 mg/kg/day dose level and between males and females therefore it is not expected that the use of a supra-MTD dose level in female rats negatively affected the ability of the study to detect drug-related neoplasms and therefore it would appear the study should be acceptable as part of the assessment for the risk of human carcinogenicity.

Evaluation of tumor findings:

Tumors which appeared to be TAK-375-related in the rat were hepatic adenoma and carcinoma in both males and females and Leydig cell tumors in male rats. These neoplasms demonstrated a dose-related increase which was statistically significant and exceeded the historical control incidence from both the lab conducting the study and the incidence found in the strain as provided by the supplier; $\bar{L}$ $\bar{1}$ Hepatic adenoma was significantly increased in males with dose levels of ≥ 250 mg/kg/day while in females this occurred with dosing ≥ 60 mg/kg/day. Hepatic carcinoma was significantly increased over control rats only with dosing at 1000 mg/kg/day. Pre-neoplastic lesions of the liver noted on histopathologic assessment included hyperplasia of the bile duct cells and proliferation of oval cells with dose levels ≥ 250 mg/kg/day in females and 1000 mg/kg/day in males. Males also demonstrated proliferation of bile duct cells at the high dose, a finding which was not identified in female rat livers. Additionally, a general altered cell focus of varying character was noted with TAK-375 levels of ≥ 250 mg/kg/day in both male and female rats. This altered hepatocellular foci finding has been described as being evidence of a pre-neoplastic lesion (Williams, 1989) but is not necessarily sufficient for the predicting the development of carcinogenicity from a regulatory standpoint in the absence of a tumor response (Maronpot et al., 1989; Moch et al., 1989). Focal necrosis and fibrosis was also much more common in rats at the high dose level when compared with controls. Examination of testicular tissue revealed hyperplasticity of Leydig cells with a clear increase above control rats apparent with the 1000 mg/kg/day dose level, the same dose at which tumors were observed.

Strengthening the validity of these findings as being drug-induced, hepatic adenoma and carcinoma was observed in the mouse carcinogenicity study as well as in the present rat study. Hepatic tumors were found in the 2-year rat study utilizing dose levels which the Sponsor has demonstrated in shorter studies of 1 month and 1 week duration are associated with an induction of hepatic enzymes. A 1-week study of the effects of TAK-375 using dose levels of 15, 60, 250 and 1000 mg/kg/day on the levels of specific hepatic drug-metabolizing enzymes was conducted (study# M-11-00593) which demonstrated that an oral dose of 250 mg/kg/day was associated with a strong induction of enzymes which resembled that seen with 60 mg/kg/day of phenobarbital, a known hepatic enzyme inducer. Enzyme induction was also found in males with doses of 15 and 60 mg/kg/day and females with the 60 mg/kg/day dose as well but these increases were much milder and occurred in fewer CYP450 subtypes. The 4-week study (study# M-11-00027) provided evidence that significant enzyme induction could occur with dosing ≥ 150 mg/kg/day in males and in females with dosing of 600 mg/kg/day.

Thus, both studies indicate the potential for development of enzyme induction, especially in long-term studies, and with males likely to be slightly more sensitive to this effect than females. As discussed in the mouse carcinogenicity study, hepatic enzyme induction such as that produced by phenobarbital is known to be associated with the development of hepatic tumors in rodents (Whysner et al., 1996) though in rats this is apparently limited to adenoma and carcinoma is not commonly observed. Nevertheless, the Sponsor provided a study which revealed an increased incidence of hepatocellular carcinoma with phenobarbital administration in rats (Parzefall et al., 1990). Thus it is plausible that the increased incidence of hepatocellular adenoma and carcinoma in rats is due to a non-specific induction of hepatic enzymes which, according to the current scientific understanding (Whysner et al., 1996) is not likely to be operative in humans due to the absence of this relationship in clinical studies.

An additional non-genotoxic mechanism for the TAK-375-associated development of hepatic tumors was provided by sponsor which involved the role of dehydroepiandrosterone (DHEA) in hepatic peroxisome proliferation and carcinogenesis. Melatonin has been shown to stimulate the release of DHEA from mouse adrenal cells *in vitro* (Haus, 1996) and oral melatonin administration has been associated with increased circulating DHEA levels in dogs *in vivo* (Ashley et al., 1999). DHEA has been shown to be a peroxisome proliferator in mice and rat liver (Frenkel et al., 1990; Yamada et al., 1991; Rao et al., 1992; Hayashi et al., 1994). DHEA has been reported to be a tumor promoter and direct hepatic carcinogen in rats (Metzger et al., 1997). Of interest, a clinical study of melatonin administration to elderly women for 6 months has shown an increase in DHEA levels (Pawlikowski et al., 2002). TAK-375 as a melatonin receptor agonist could conceivably produce an increase in circulating DHEA resulting in hepatic peroxisome proliferation and subsequent development of neoplasms though this hormone has also been associated with hepatic enzyme induction as well (Metzger et al., 1997). Perhaps notably, a description of changes consistent with peroxisome proliferation was not specifically noted in the histopathologic report. A separate study (study# M-11-00599) was conducted to determine the effect of TAK-375 on plasma concentrations of melatonin, DHEA and correlative ultra-structural changes in hepatocytes with oral administration of doses used in the rat carcinogenicity study for 4-weeks. Results of this study revealed a TAK-375-related increase in plasma melatonin levels with ≥ 60 mg/kg/day dosing within 1 hr of administration while at 250 mg/kg/day melatonin levels were increased at both 1 hr and 15 hr time-points. DHEA levels were increased with a dose level of ≥ 250 mg/kg/day at 1 hr post-administration while levels were increased at both 1 and 8 hr with 1000 mg/kg/day dosing. Proliferation of the smooth endoplasmic reticulum was confined to the 1000 mg/kg/day dose level which is consistent with hepatic enzyme induction but no changes in the peroxisome characteristics was observed using electron microscopic analysis. Assessment of liver tissue for expression of proliferating cell nuclear antigen (PCNA), indicative of hepatic proliferation, revealed an increase which did not rise to the level of statistical significance with the 1000 mg/kg/day dose. The activity of acyl-CoA oxidase, a marker enzyme for peroxisome proliferation, did not increase in any tested group suggesting that at least with this short-term administration of TAK-375 that a peroxisome proliferation mechanism underlying tumor formation is unlikely while hepatic enzyme induction as an epigenetic mechanism for tumor development is more probable.

Leydig cell tumors of the testes were observed to be significantly increased above control animals only with the 1000 mg/kg/day dose level while the 250 mg/kg/day level was associated with a non-significant increase in incidence. An apparent increased incidence of Leydig cell hyperplasia was observed in the 250 mg/kg/day dose group and above (note: statistics not used for evaluation of non-neoplastic lesions in the present study). Leydig cell tumors in rodents have been associated with disruption of normal hypothalamic-pituitary-gonadal axis homeostasis, for example aromatase or testosterone synthesis inhibitors, compounds which enhance GnRH release or are GnRH agonists, etc. The ultimate change in hormonal levels usually involves an increase in serum leutenizing hormone (LH), which is involved in stimulating testosterone production from Leydig cells. Alternatively, induction of Leydig cell tumors has been ascribed to an increase in sensitivity to circulating LH. The effect of TAK-375 on plasma levels of circulating testosterone and LH in rats treated for 1 week was assessed in two studies (M-11-00689 and M-11-00690) using identical methodologies but different sampling time-points and subsequently a 4-week study (M-11-00691) was conducted as well. Results from the 1-week studies in male rats clearly indicated that oral administration of TAK-375 in a dose of 250 mg/kg/day or 1000 mg/kg/day significantly suppressed plasma testosterone levels for up to 4 hr after the last dose but returned to normal by 6 hr. No alteration in circulating LH levels was observed at any dose or time-point, however. The 4-week oral study of TAK-375 was conducted to determine if a longer duration of administration would detect a more subtle change on LH levels that might point towards this non-genotoxic mechanism of Leydig cell tumorigenesis. As in the 1-week studies, rats (n = 40-50 males/group) were administered vehicle, or TAK-375 at doses of 250 or 1000 mg/kg/day for 14 and 28 days prior to assessment. In agreement with the previous studies, TAK-375 in doses of 250 mg/kg/day and greater were associated with a decrease in plasma testosterone levels which was significant maximally for 4 - 5 hr after final administration at the 2-week and 4-week assessment. In contrast to the 1-week studies, however, circulating LH levels appeared to be slightly but significantly increased at the 4.5 hr time-point with 1000 mg/kg/day dosing ($\uparrow 63\%$) and 24 hr time-point at 250 ($1.86 \pm .51$ ng/mL; $\uparrow 77\%$) and 1000 (1.67 ± 0.25 ng/mL; $\uparrow 59\%$) mg/kg/day dose levels relative to control animals ($1.05 \pm .77$ ng/mL). Thus it seems likely that a TAK-375 (or the active metabolite M-II, or both compounds) inhibition of testosterone and consequent elevation in LH levels, though transient in nature, may underlie the observed Leydig cell tumors in the rat. Though this mechanism may be operative in man (i.e. reduced testosterone and or increased circulating LH), there is evidence that the Leydig cells of humans are significantly less sensitive to elevations in plasma LH than exhibited in the rat model and are therefore unlikely to be a significant risk factor for adult males in the course of typical treatment. Several compounds which are known to produce Leydig cell tumors in rats (ex: 1,3-butadiene, cadmium, ethanol, lactose, lead, nicotine) are not associated with development of these tumors in man with typical exposures to these chemicals (Cook et al., 1999).

As discussed previously, these epigenetic explanations may not be required as TAK-375 and/or its metabolites have demonstrated evidence of genotoxicity in an *in vitro* chromosomal aberration assay both with and in the absence of metabolic activation. Though the results of several additional *in vitro* studies and *in vivo* micronucleus assays in mouse and rat were judged negative, a subsequent *in vivo/in vitro* Unscheduled DNA Synthesis assay conducted in rat liver tissue was considered positive by the reviewer, Dr. Aisar Atrakchi (see GENETIC

TOXICOLOGY section). Therefore the potential remains that the tumors associated with TAK-375 exposure in the 2-year rat bioassay are due to a direct genotoxic effect of the parent drug and/or a metabolite.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: Sprague-Dawley rats administered TAK-375 with doses of 0, 15, 60, 250 or 1000 mg/kg/day for 104-weeks by oral gavage demonstrated a dose-dependent and severe reduction in body weight associated with excessive mortality. This decrease in survival required the early termination of the high dose female group which, along with body weight reduction, indicated the MTD was exceeded at the high dose in both males and females. Nevertheless, a consistent dose-dependent increase in incidence of hepatic tumors was observed in both male and female rats and Leydig cell tumors in males. With inclusion of the 1000 mg/kg/day group data the findings in males included hepatic adenoma, carcinoma and hepatoblastoma as well as Leydig cell tumors. In females the findings included hepatic adenoma and carcinoma. With the exclusion of the 1000 mg/kg/day group data in males only hepatic tumors demonstrated a significant trend while in females only hepatic adenoma was significantly increased above controls. Extrapolation of toxicokinetic data to the positive tumor findings would suggest a $\geq$ 1,419-fold margin of safety for humans over hepatic tumors and a nearly 4,472-fold margin for Leydig cell tumors when considering TAK-375 plasma concentrations at the NOAEL dose. When considering the M-II exposure, however, these margins drop to $\geq$ 12-fold and 14-fold for hepatic tumors and Leydig cell tumors, respectively. The Sponsor argues that the hepatic tumors are due to induction of hepatic metabolizing enzymes and this is not likely to be operative in humans, a finding which is plausible. The Sponsor further argues the Leydig cell tumors are specific to rat as reductions in testosterone and an increase in LH has been demonstrated in shorter toxicology studies with repeated administration of TAK-375 while the Leydig cells of human are thought less sensitive to elevations of LH, should this occur, and therefore this tumor would not be expected to develop. This also appears to be plausible.

Conclusions and Recommendations: The MTD level was exceeded in this study in both males and females though this does not qualitatively alter the findings of hepatic tumors both males and females and Leydig cell tumors in males. While highly plausible mechanistic arguments were made which suggest these findings are unlikely to be found in humans it is noted that several genotoxicity studies performed by the Sponsor were judged positive. Thus the findings may not be indicative of an epigenetic mechanism but instead could be due to a direct genotoxic effect of the test article.

Reviewer Signature Adam M. Wasserman, Ph.D.

Supervisor Signature R. Daniel Mellon, Ph.D. Concurrency Yes No

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APPENDIX/ATTACHMENTS

Appendix 1: ECAC meeting minutes dated May 9, 2000

Appendix 2: Review of Amendment #024 by Aisar Atrakchi, Ph.D. dated February 13, 2002

Appendix 1: Executive CAC Meeting Minutes

Executive CAC

Date of Meeting: May 9th 2000

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Albert DeFelice, Ph.D., HFD-110, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Team Leader

Author of Draft: Aisar H. Atrakchi, 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. The committee did not address the sponsor's statistical evaluation for the 2-yr carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the studies. The sponsor may seek guidance on the statistical evaluation of the bioassay results from Agency staff separately.

IND #: 58,136
Drug Name: TAK375
Sponsor: Takeda America, Inc.
Princeton, NJ 08540

BACKGROUND:

TAK 375 IS A MELATONIN AGONIST DEVELOPED AS THE S-ENANTIOMER WITH VERY LOW SOLUBILITY IN WATER AND OTHER AQUEOUS SOLUTIONS WITH PH 3-11. TAK 375 SHOWED HIGH AFFINITY TO MLI RECEPTORS WITH KI PF 25.4PM IN CHICK FOREBRAIN ASSAY COMPARED WITH MELATONIN KI OF 399 PM IN THE SAME ASSAY. THIS DRUG IS INTENDED TO TREAT SLEEP DISORDERS. THERE IS NO CLINICAL EXPERIENCE EXCEPT FOR A SINGLE DOSE PHASE I STUDY IN JAPAN THAT IS ONGOING; DATA NOT YET AVAILABLE.

Comment:

Note that the mouse and rat carcinogenicity studies are ONGOING (initiated in Nov. 1999). The sponsor requested Executive CAC comments/input on the protocols and selection of doses.

Rat Carcinogenicity Study and Dose Selection

The doses in the 2-year carcinogenicity study are 15, 60, 250, 1000 mg/kg/day. The sponsor selected these doses based on MTD obtained from 26-wk oral gavage tox/TK study where the top dose was 150 mg/kg/d. In addition to this 6-month study, there are two 1-month and one 13-week oral tox/TK studies with doses up to 1500 mg/kg/d*. Death occurred at 1500 mg/kg/d but not at 1200 mg/kg/d. Histopathological findings were observed at ≥ 150 mg/kg/d in liver, adrenals, thyroids, kidneys, and ovaries. Some of these findings are considered by the reviewer to be extensions of pharmacology of the drug (melatonin agonist). In the current 26-week oral gavage dose selection study, there were no deaths up to 150 mg/kg/d. Organ weight changes and histopathology were seen in liver, adrenals, thyroids, ovaries, and spleen, similar to the other subchronic toxicity studies in the rat. The NOAEL in the 26-week dose selection study is 40 mg/kg/d and the MTD is $\gg 150$ mg/kg/d but may be < 1500 mg/kg/d based on observation of acute lethality.

** Complete reports for these studies were not part of this submission; the reviewer requested them and were later submitted by the sponsor. These studies consequently have been reviewed.*

Mouse Carcinogenicity Study and Dose Selection

The doses in the 2-year carcinogenicity study are 30, 100, 300, 1000 mg/kg/day. These doses were selected based on MTD obtained from 13-wk oral gavage tox/TK study at doses up to 1000 mg/kg/d. From a previously conducted 1mo oral tox/TK study in mice, death occurred at the highest dose of 1500 mg/kg/d therefore, the top dose in the 13-week study was selected as 1000 mg/kg/d. Following 2 days of dosing at 1000 mg/kg, 1 male died 1hr post dose. At 300 mg/kg/d the only findings were increase in absolute and relative liver weights in males and histopathology indicative of enzyme induction. Therefore, MTD is >>300 but may be <1000 mg/kg/d based on acute lethality.

Executive CAC Recommendations and Conclusions:

Rat study: The committee could not concur on the doses selected for the ongoing carcinogenicity study based on the available information. This is because the highest dose in the 26-week dose range finding study of 150 mg/kg/d does not appear to define the MTD, since the findings at this dose were mild and considered extensions of the pharmacology of the drug (melatonin agonist). In 1 month oral tox study death occurred at 1500 mg/kg/d but no death and no marked or direct drug effects were observed at 1200 mg/kg. Therefore, the MTD appears to be <1500 mg/kg/d, however, this can be more readily determined based on the observations of the ongoing carcinogenicity study. Information from the ongoing study will be needed before concurrence can be offered.

Mouse Study: The committee could not concur on the doses selected for the ongoing carcinogenicity study because at the top dose of 1000 mg/kg/d, death occurred at this dose in the 13-week dose range finding study suggesting that the MTD could be exceeded. The next lower dose is 300 mg/kg/d at which no marked or direct adverse drug effects were observed. Therefore, the MTD may be <1000 mg/kg but >>300 mg/kg/d. Observations from the ongoing carcinogenicity study can address this and will be needed prior to offering CAC concurrence.

For both the rat and mouse studies, the committee recommends that the sponsor review the preliminary data from the now 6-month ongoing carcinogenicity studies and determine if the doses selected seem appropriate based on parameters that can be evaluated at this time of the study such as changes in body weight, food intake, extent and frequency of clinical signs and, mortality. After this assessment, the sponsor may provide this information to the Division if concurrence on dose selection is desired.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\ IND /Division File, HFD-120
AM. Hommonay, HFD-120
G. Fitzgerlad, HFD-120
Atrakchi, HFD-120
A. Seifried, HFD-024

Appendix 2: Review of Amendment #024 by Aisar Atrakchi, Ph.D.**Pharmacology Toxicology Review
Amendment# 024**

IND# 58,136
Drug: TAK 375
Sponsor: Takeda Pharmaceuticals America, Inc.
Lincolnshire, IL 60069
Category: Melatonin Agonist
Indication: Hypnotic
Sub Date: Oct 3rd 2000
Rec Date: Oct 5th 2000
Rev Date: Feb 13th 2002
Reviewer: Aisar H. Atrakchi, Ph.D.
Team Leader: Barry Rosloff, Ph.D.
Related END/NDAs: NA

This submission contains 2 reports#s M-11-247 and M-11-243. These reports represent responses to the Executive CAC meeting minutes dated May 9th 2000 on rat and mouse carcinogenicity protocols. The carcinogenicity studies were ongoing for several months when the sponsor submitted their protocols for concurrence from the Executive CAC. The Executive CAC could not concur on the doses for either the rat or mouse carcinogenicity studies due to lack of adequate information. The sponsor in this submission provided specific response to the Executive CAC comments (# M-11-247) and, an interim summary report (26wk) of the rat 2 yr oral gavage carcinogenicity study (# M-11-243). The sponsor requested review and comments on these 2 reports specifically on dose selection and the results of the 26wk interim sacrifice.

The Division can not provide concurrence or any additional advice to the sponsor since the carcinogenicity studies in the rat and mouse are/were ongoing. The sponsor at this time has to use their judgement to determine if the doses selected for the carcinogenicity are appropriate based on a number of study parameters that were outlined in the Executive CAC meeting minutes of May 2000.

cc.

Div. File Orig. IND# 58,136

B. Rosloff, A. Atrakchi, M. Shin.

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

/s/

Aisar Atrakchi
2/14/02 08:59:50 AM
PHARMACOLOGIST

Barry Rosloff
2/15/02 03:24:27 PM
PHARMACOLOGIST

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Effects on fertility and early embryonic development to implantation in rats.

Study No: M-11-37

Site and testing facility: Drug Safety Res Labs, Takeda Chemical Industries, Osaka, Japan.

GRP compliance: Yes/Japanese & FDA

QA- Reports Yes (x) No ()

Study initiation/completion: Mar 1998/Nov 1998

Lot and batch numbers: M-375-003

Protocol reviewed by Division Yes () No (x)

Stability/purity: Stable throughout dosing^a —

Methods:

- Species/strain: Spargue-Dawley rats.
- Doses employed: 6, 60, 600mg/kg/d; 10ml/kg
- Route of Administration: oral gavage.
- Study Design: doses selected based on a previous 2wk tox study in rats at doses 30, 200, 600, and 2000mg/kg/d and a dose range finder in rats to test effects on embryo-fetal development at doses 10, 100, and 1000mg/kg/d. Deaths occurred at the highest doses of 2000 and 1000mg/kg in both studies, at 600&2000mg/kg there was a decrease in mean wt and food intake as well as ataxia. Therefore, 600mg/kg/d dose was selected for the present study as the high dose. In the present study, males were treated once daily starting 4wks prior to mating and continuing through mating till the females were necropsied on gd13; females were dosed 2wks prior to mating and continued through gd6. The control gr received 0.5%w/v MC solution following the same schedule as the drug grs.
- Number of animals/sex/dosing group: 20/sex/dose plus 20/dose untreated females for the 2nd mating.
- age/weight: 11wk old males and 10wk old females/371-405g m and 223-241g f.
- Parameters and endpoints evaluated:
 - ❖ survival & signs: 3x/d prior to dose, immediately after dose, and 0.5-2hr postdose throughout the study duration in addition. females were observed 1x per day during non-dosing period.
 - ❖ B.wt: m+f 2x per wk throughout the study for m and. for f before mating and on days 0,3,7,10,&13 of gestation; untreated f were not weighed.
 - ❖ food intake: one day consumption done for all treated rats (m+f) 1x per wk before mating and for pregnant f on days 0,3,7,10, &12 of gestation. non-pregnant f not measured.
 - ❖ For the 1st mating: estrous cycle, copulatory index, fertility index, macroscopic exam of pregnant females killed on gd13 for repro parameters included: uterus, ovaries, # of corpora lutea, implants, dead embryos (resorptions, dead and live embryos), wt of liver & adrenals (absolute and relative) for each dam, and liver/adrenals/uterus/ovaries & live embryos were fixed in 10%v/v formalin for examination.
 - ❖ For the 2nd mating: following 7wk of dosing, treated males in control, 60, and 600mg/kg were mated for 2wks with untreated females. The following were assessed: copulatory interval, copulatory index, & fertility index.

- ❖ For the 2nd mating, all pregnant/untreated females were killed on gd13 and the following examined: macroscopic exam of uterus, ovaries, # corpora lutea, # implants, dead and live embryos, and the uteri, ovaries, and live embryos were fixed in 10%w/v formalin for examination.
- ❖ Males treated for a total of 9wks were weighed then killed, their reproductive and main organs were examined macroscopically. Weights were taken for testes, epididymides, seminal vesicles, ventral prostate, liver and adrenals. Testes and epididymides were fixed in Bouin's solution for 2-3days then fixed in formalin, the other organs were fixed in formalin. Testes, epididymides, prostate, and seminal vesicles were examined histologically using H&E stain. Sperm motility, count, and morphology were done on each male (400 sperms per male were examined for morphological changes under the microscope).
- Statistical evaluations: Fisher's exact test, Dunnett type test, and Bartlett's test for homogeneity of variance.

Results:

- Clinical signs: were seen exclusively in HD and included in males (most common signs): decreased spontaneous activity (17 out of 20), chromaturia (20/20), salivation (20/20); less common signs were ataxia, creeping, crouching, decrease of fecal pellets, and ptosis. In females pre-mating: lying on side (11/20), bradypnea (10/20), decreased fecal pellets (10/20), salivation (12/20), during mating and gestation: salivation (7/20). Other signs were of low incidence and included: creeping, crouching, low body temp., ataxia, no feces, and loss of incisor.
- Mortality: 9/20 females dosed 600mg/kg died after the 1st, 2nd, or 3rd dose. prior to death, these animals showed creeping, lying on side, lacrimation, bradypnea, decreased spontaneous activity, decreased body temp, and absence of feces. Similar signs were also seen in surviving animals (m and f) in the high dose.
- Body weight: mean wt and wt gain were significantly reduced in males as early as 3days after start of dosing and lasted throughout the dosing period in males and through gestation in females of 600mg/kg dose. The decline in mean wt ranged between 3-8% less than the control in males and 4.6-9% in females relative to the control.
- Food consumption: significantly decreased on d1 of dosing in males (31% lower than control) and in females on gd3 12% of control.
- Toxicokinetics: not done in this study.

Fertility and Early Embryonic Development in Females:

- In-life observations:
 - ❖ frequency (number) of irregular estrous cycles was increased in females during gestation at 10/20 dams in the 60mg/kg gr and 6/11 in the 600mg/kg gr vs. 0 in the control or 50&54.6% respectively, as percent of irregular cycles relative to control.
 - ❖ No effect on fertility or copulatory indices of both mating periods.
- Terminal and Necroscopic evaluations: in the 9 high dose dams that died shortly after dosing, petechia in stomach, liver & spleen discoloration, enlarged adrenals, and congested lungs were observed. C-section on gd13 of all surviving dams showed significant increase in mean wts of the liver (18&26% for absolute and relative wts respectively) and adrenals (19&29% over the control for absolute and relative wts respectively), and a significant decrease in number of corpora lutea in the 600mg/kg gr. Mean number of implants was reduced in 60&600mg/kg grs (significant only in HD), mean pre- and post-implantation loss were increased in 60mg/kg but no significant finding in HD. Mean number of live embryos was reduced in 60&600mg/kg grs (table from sponsor). Note that for the pre- and post-implantation loss, there was a great deal of inter-individual variation as seen from the large s.d (see reviewer table). None of these changes were seen in the untreated females of the 2nd mating although large inter-individual

variation was also evident for the 60&600mg/kg grs specially for pre- and post-implantation loss relative to the control.

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Table 15 Necropsy findings in dams and embryos - On day 13 of gestation
 - The first mating (Treated male x Treated female)

Group	1		2		3		4	
Compound	Control		TAK-375		TAK-375		TAK-375	
Dose(mg/kg/day):	-		6		60		600	
Group	1		2		3		4	
No. of animals	19		20		10		10	
Mean no. of corpora lutea (A)	16.4±	1.6	16.6±	2.2	15.4±	1.8	14.1±	1.9**
Mean no. of implants (B)	15.1±	1.7	15.5±	1.4	12.1±	5.0	13.0±	1.8*
Mean pre-implantation loss (A-B)/(A) (%)	7.6±	9.8	5.8±	6.6	22.0±	31.2	7.6±	5.6
Mean no. of dead implants								
Resorbed	0.0±	0.0	0.0±	0.0	0.0±	0.0	0.0±	0.0
Placental remnants	0.5±	0.6	0.7±	0.7	0.8±	0.6	0.5±	0.7
Dead embryos	0.0±	0.0	0.1±	0.3	0.1±	0.2	0.1±	0.3
Total (C)	0.4±	0.6	0.8±	0.9	0.9±	0.6	0.6±	0.7
Mean post-implantation loss (C)/(B) (%)	2.8±	4.0	5.0±	6.0	12.7±	22.1**	4.8±	5.7
Mean no. of live embryos	14.7±	1.7	14.8±	1.8	11.2±	5.0*	12.4±	2.0*

Significantly different from group 1 (*:p≤0.05 or **:p≤0.01)

Table below presents Range of values from individual data for the following parameters from the 1st mating:

- 60mg/kg:n=20
 - 4 dams had 1, 4, 7, 9 # of implants
 - 5 dams had pre-implantation loss outside the range of the control (47, 50, 71, 83, 93.8% of control)
 - 4 dams had post-implantation loss greater than the control with 1 dam had lost its entire litter (100% of control). Total post-implantation loss was 17 and mean percent was 12.7+22.

- 600mg/kg:n=10
 - all 10 dams had # of implants that were on the low end of the range of those in the control (in 600mg/kg: single dams at 10, 11, 12, 14, 15, and 16; and four dams at 13 implants). Total post-implantation loss was 6 vs. 8 in control but the mean percent (total loss / # of implants) gave 4.8-5.7 vs. 2.8-4.0 in the control.

- Control:
 - the # of implants ranged between 13-17 per dam with 1 dam having 18 and another dam having 12 implants. 5 dams had pre-implantation loss of 10.5, 11, 27.8, 36.8, and 12.5% (# corpora lutea # of implants), the rest of the dams showed very little or no loss. Total post-implantation loss was 8 and mean % was 2.8+4.0.

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Necropsy findings and organ wts in males: dark and enlarged livers of 600mg/kg gr was seen in 11&12 of 20 animals also the adrenals were enlarged in 3/20. Significant increases noted in absolute and relative wts of the liver, adrenals, testes and epididymides (these last 2 organs only relative wt was significantly increased) in the 600mg/kg gr all relative to the corresponding control values (see table below). Note that mean body wt in the 600mg/kg was significantly reduced therefore, these changes in mean organ wts may be secondary to the reduction in body wt thus not direct drug effect.

There were no drug related effects on sperm morphology, motility, or count in any drug group and no histopathological findings in the male reproductive organs.

Organ wts in males relative to control presented as percent of body wt, values are means±s.d.

Organ		Control	600mg/kg/d
Liver		3.3±0.2	5.4±0.4*
Adrenal gland		8.3±1.4	13.6±2.2**
Testis	L	0.32±0.03	0.35±0.03**
	R	0.32±0.03	0.35±0.03
Epididymides	L	115±9	126±11*
	R	118±9	127±11*

* p<0.05

**p<0.01 difference from the control

Summary & Evaluation:

TAK375 was orally administered to males 4wks prior to mating and through the day before necropsy and for 2wks to females prior to mating through gd6, drug effects on fertility and reproductive performance of males and females were assessed. The doses tested were 6, 60, and 600mg/kg/d prepared in 0.5% MC administered by oral gavage. The NOEL in males was 60mg/kg (due to changes in organ wts at 600mg/kg), in females was 6mg/kg (due to irregular estrous and effects on # of corpora lutea, pre- and post-implantation loss, and # of live fetuses at 60mg/kg), and the NOEL of the drug on fertility and reproductive performance is 600mg/kg for the male and females.

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Death in 600mg/kg gr occurred in 9/20 pregnant females early in the study (days 1-3 of dosing), no deaths in males. Clinical signs were seen exclusively in HD included creeping, lying on side, lacrimation, bradypnea, decreased body temperature, absence of feces, chromatorea, and reduced spontaneous activity. Mean body wt and wt gain were significantly reduced in males and females of 600mg/kg, food intake was reduced significantly but only during early dosing period. There was an increase in the number of irregular estrous cycles in HD relative to the control, it is known though that administration of melatonin affects estrous cycle therefore, this effect *maybe* related to the pharmacological effects of the drug. A significant increase in mean pre- and post-implantation loss were seen in 60mg/kg (though no effect in HD), significant decrease in # of implants and # of corpora lutea in 600mg/kg gr (# of implants was also reduced in 60mg/kg gr but not significantly), mean number of live embryos was reduced in 60&600mg/kg grs. Noted that in the 2nd mating of treated males to untreated females, none of these findings were seen in any drug group but large interanimal variations was present. There was no effect on fertility or copulatory indices of both mating periods. Mean absolute and relative wt of the liver and adrenals of both males and females were significantly increased in HD, not accompanied by histopath findings. There were no drug effect on sperm count, motility, or morphology.

Study title: Effects on fertility and early embryonic development to implantation in rats/Supplemental study.

Study No: M-11-38

Site and testing facility: Drug Safety Res Labs, Takeda Chemical Industries, Osaka, Japan.

GRP compliance: Yes: Japanese & FDA

QA- Reports Yes (x) No ()

Study initiation/completion: May 1998/Oct 1998

Lot and batch numbers: M-375-003

Protocol reviewed by Division Yes () No (x)

Stability/purity: Stable throughout dosing —

Methods:

- Species/strain: Sprague-Dawley rats.
- Age/wt. males: 6- & 11-wk. females: 6- & 12wks/wt in males age 6wks: 192-195g/males age 11wks: 360-383g; females age 6wks: 148-154g and females age 12wks: 244-261g.
- Doses employed: for the 11/12wk old rats: 6, 60, 600mg/kg/d. for the 6wk old rats: 60&600mg/kg. Dose volume 10ml/kg.
- Route of Administration: oral gavage.
- Number of animals/sex/dosing group: see table below from sponsor:

Group	Test article	Dosage level (mg/kg)	Dosage volume (mL/kg)	Concentration of dosing suspension (w/v%)	No. of animals	
					Males	Females
1	TAK-375	6	10	0.06	4 ^{a)}	4 ^{b)}
2	TAK-375	60	10	0.6	4 ^{a)}	4 ^{b)}
3	TAK-375	600	10	6	4 ^{a)}	6 ^{b)}
4	TAK-375	60	10	0.6	4 ^{c)}	4 ^{c)}
5	TAK-375	600	10	6	4 ^{c)}	4 ^{c)}

a: 11 weeks old, b: 12 weeks old, c: 6 weeks old

Study Design: This study was done in attempt to address the reason(s) for the differences in findings between female rats in the Segment I/fertility study and the 4wk oral tox study where death was seen in the former study but not in the latter at the same doses. The present study was a single dose administration and the animals killed on the day post dose. The doses were 6, 60, and 600mg/kg as in the above table, the age of the animals were selected to cover those used in the 4wk tox study (6wks) and those used in the fertility study (m:11wks and f 12wks). All animals were observed for clinical signs predosing and at 0.5-2hr postdose. Blood was collected from the tail vein at 0.25, 0.5, 1, 3, 6, and 24hr postdose. Gross exam was also done.

Results:

One female rat aged 12wks and dosed 600mg/kg died during final blood collection and another female was killed in extremis after the last blood sample was collected. No other deaths occurred in any group including the 6wk old females dosed 600mg/kg. Clinical signs included creeping, crouching, decreased spontaneous activity or ataxia seen in the 600mg/kg rats of the 3 age groups (11/12wk and 6wks). However, the signs were more severe in the older animals. Post-treatment signs in the 11/12wk old rats dosed 600mg/kg were decreased feces, lying on side, bradypnea, low body temp., and lacrimation. In the 6wk old rats of 600mg/kg post-treatment signs included absence of feces and decreased spontaneous activity.

Table below summarizes the TK data:

Table 1 Toxicokinetic parameters for TAK-375 in 11- and 12-week-old rats

Dose (mg/kg)	Male ^a (n=4)			Female ^b (n=4)		
	T _{max} (h)	C _{max} (µg/mL)	AUC _{0-24h} (µg·h/mL)	T _{max} (h)	C _{max} (µg/mL)	AUC _{0-24h} (µg·h/mL)
6	0.4 (0.1)	0.40 (0.09)	0.6 (0.1)	0.3 (0.1)	1.24 (0.64)	1.3 (0.3)
60	0.4 (0.1)	8.74 (1.94)	26.3 (10.0)	0.6 (0.3)	15.13 (3.50)	67.8 (13.5)
600	1.3 (1.2)	28.66 (5.82)	230.5 (64.3)	3.8 (2.5)*	78.50 (49.15)*	1,253.1 (998.1)*

Table 2 Toxicokinetic parameters for TAK-375 in 6-week-old rats

Dose (mg/kg)	Male (n=4)			Female (n=4)		
	T _{max} (h)	C _{max} (µg/mL)	AUC _{0-24h} (µg·h/mL)	T _{max} (h)	C _{max} (µg/mL)	AUC _{0-24h} (µg·h/mL)
60	0.4 (0.1)	8.38 (1.93)	15.3 (4.2)	0.5 (0.0)	11.30 (2.39)	38.3 (11.1)
600	0.6 (0.3)	27.21 (7.06)	178.2 (30.8)	2.3 (2.5)	51.53 (29.64)	601.9 (521.6)

Mean (S.D.)

^a: Male, 11-week-old ; Female, 12-week-old

*: n=6

The ranges of C_{max} and AUC values are as follows:

Dose (mg/kg)	C _{max}		AUC _{0-24h}	
	11/12wk	6wk	11/12wk	6wks
60	m 0.06-11.6	0.17-11.4	m 17-40.5	10-19.7
	f 1.9-18.6	0.37-13	f 50-71.5	24.6-51
600	m 0.11-30.6	0.02-30	m 178-318	149-221
	f 4-154.3	0.08-92	f 380.5-2530	305-1363

The data shows at both doses, no difference in max concentration (except as below), or mean T_{max} values at 60mg/kg for both sexes between the 2 age grs. However, exposure at both doses, C_{max} in females at 600mg/kg, and T_{max} at 600mg/kg for both sexes, were lower in the 6wk old rats relative to the corresponding values in the older rats. Therefore, the sponsor concluded that the differences in response (presence and absence of mortality) between the 4wk tox and Segment I fertility studies in the rat, is differences in plasma concentration and exposure between the 2 age groups at the same doses i.e higher levels in Segment II where 9f were dead at 600mg/kg than rats in the 4wk tox study dosed 600mg/kg where no deaths were seen.

Note that the sponsor did not explain why such differences were seen, did absorption improve in the older rats, did the drug clear faster in younger animals, was it both factors, or were there other reasons.

Study title: Effects on fertility and early embryonic development to implantation in rats/Supplemental study II.

Study No: M-11-74

Site and testing facility: Drug Safety Res Labs, Takeda Chemical Industries. Osaka, Japan.

GRP compliance: Yes/Japanese & FDA

QA- Reports Yes (x) No ()

Study initiation/completion: Jul 1998/Nov 1998

Lot and batch numbers: M-375-006 (different from the above 2 studies).

Protocol reviewed by Division Yes () No (x)

Stability/purity: Stable throughout dosing —

Methods:

- Species/strain: Sprague-Dawley rats.
- Age/wt: 11wk old males and 10wk old females/380-406g m and 223-235g f.
- Doses employed: 20, 60, 200mg/kg/d. pregnant females were dosed from 2wks pre mating through gd6 and males dosed 4wks pre mating and continued till day before necropsy (i.e. about 7wks of dosing).
- Route of Administration: oral gavage.
- Number of animals/sex/dosing group: 20/sex/dose; dose volume 10ml/kg at concentrations of: 0.2, 0.6, and 2w/vol% for the 20, 60, and 200mg/kg doses respectively.

Comments:

This study was done to determine if the reproductive findings seen in the previous study were drug related (#M-11-37). Note that the low and high doses in this study were not the same as those in the previous study (lower doses used in this study), therefore, making a direct comparison difficult.

Parameters:

Mortality, clinical signs, B.wt. food intake, estrous cycle, mating performance (mating index, fertility index, copulatory interval), necropsy in dams, and necropsy and organ wts in males.

Results:

Mortality & Clinical signs: no deaths in any gr. Clinical signs were similar to those seen in previous studies and included: creeping, crouching, and hypoactivity in both males and females dosed 200mg/kg (after the 1st dose in males and only after few doses in females). Salivation was seen in almost all males and females dosed 200mg/kg. Females dosed 200mg/kg had reduced feces during early period of dosing. No signs were seen in the 20&60mg/kg groups.

B.wt & Food intake: no drug related effects in males. In females dosed 200mg/kg a significant ($p \leq 0.05$) decrease in mean wt gain was seen on days 3, 7, 10, and 13 of gestation and mean wt gain was significantly reduced on pre-mating days 14-17 and 24-28 and on gestation days 0-3 ($p \leq 0.01$) and 7-10; all compared to the control vehicle group. No drug related effects on food intake in any group.

Estrous cycle: the # of irregular cycles was higher in the 60&200mg/kg groups relative to the control (3, 1, 6, 6 in control, 20, 60, and 200mg/kg groups respectively; % of irregular cycles were: 15, 5, 30, and 30% respectively).

Mating behavior: no drug effect on copulatory index. The mean copulatory interval was highly variable: 2.9±3.0, 4.1±4.4, 2.8±1.2, and 2.2±1.0 days in control, 20, 60, and 200mg/kg groups respectively. The number of fertile groups was 20, 19, 19, and 17 in the control and drug groups respectively, which translates to fertility index of 100, 95, 95, and 85% respectively (slightly reduced in 200mg/kg group). The sponsor stated that this fertility index was not drug related since it is within the historical range of the lab at 77.8-100%.

Necropsy of Dams: necropsy was done on gd13 and no drug related effects were seen in any group. A slight but significant increase in # of implants were seen in 60mg/kg group compared to the control (table below from sponsor). There was a large interanimal variation in the postimplantation loss in all groups including the control.

Table 12 Necropsy findings in dams and embryos - On day 13 of gestation

Group	1	2	3	4
Compound	Control	TAK-375	TAK-375	TAK-375
Dose (mg/kg/day)	-	20	60	200
Group	1	2	3	4
No. of animals	20	19	19	17
Mean no. of corpora lutea (A)	16.2± 1.0	16.3± 1.5	16.9± 1.5	15.8± 1.2
Mean no. of implants (B)	14.7± 2.4	15.7± 1.4	16.2± 1.7*	15.1± 1.1
Mean pre-implantation loss (A-B)/(A) (X)	9.3± 13.5	3.7± 6.0	4.4± 5.4	4.3± 5.0
Mean no. of dead implants				
Resorbed	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
Placental remnants	0.5± 0.5	0.8± 1.0	1.3± 0.9*	1.1± 0.9
Dead embryos	0.1± 0.3	0.1± 0.2	0.0± 0.0	0.0± 0.0
Total (C)	0.6± 0.6	0.8± 1.0	1.3± 0.9	1.1± 0.9
Mean post-implantation loss (C)/(B) (X)	4.1± 4.2	5.2± 6.2	7.7± 5.7	7.1± 6.1
Mean no. of live embryos	14.1± 2.4	14.8± 1.5	14.9± 1.7	14.1± 1.6

Significantly different from group 1 (t: $p \leq 0.05$ or $p \leq 0.01$)

Necropsy and organ wts in males: no consistent or drug related findings were seen in any group. The following were changes in organ weights that were not accompanied by histopath. Mean absolute and relative wt of the ventral prostate was reduced non dose dependently in all 3 male groups with statistical significance only in 60mg/kg group, relative to the control (at 60mg/kg the relative wt was 86% of control). Mean absolute and relative of the seminal vesicles was reduced significantly but not-dose dependently in both 60&200mg/kg'd groups (relative wts were 84&89% of control respectively).

Summary and Conclusions:

In the previous study (#M-11-37), there was an increase in the number of irregular estrous cycles relative to the control, a significant increase in mean pre- and post-implantation loss at the 60mg/kg (though no effect at 600mg/kg), a significant decrease in # of implants and # of corpora lutea in 600mg/kg gr (# of implants was also reduced in 60mg/kg gr but not significantly), and the mean number of live embryos was reduced in 60&600mg/kg grs. Except for the increase in number of irregular estrous cycles, none of the other findings were observed in the current study at the 60mg/kg dose which is the common dose in both studies. The sponsor referred to Chu et al., 1962 publication where injection of melatonin decreased the incidence of estrous in mice and rats and proposed that endogenous melatonin play a role in regulation of estrous cycle in rodents. Ying & Greep 1973 suggested that melatonin inhibits LH release inhibits ovulation in rats. Based on these articles, and that TAK-375 is a potent melatonin-1 agonist, the sponsor suggests that the effects on estrous cycle in the 2 studies are extensions of the pharmacological effects of the drug. The NOAEL in males is 60mg/kg/d and 20mg/kg/d in females. The drug did not affect fertility parameters in males upto 200mg/kg dose. in females the NOAEL is <60mg/kg/d due to the effects on estrous cycle in this study and other reproductive parameters in the previous study.

Summary and Conclusions for the Reproductive Studies:

Oral gavage administration of TAK375 to male and female rats (before and during pregnancy), caused death early in the study in females dosed 600mg/kg/d and clinical signs. The sponsor attempted to address why deaths were seen in the repro studies when no deaths occurred in tox studies at 600mg/kg dose. The sponsor concluded that the concentration and exposure to the drug were much higher in rats used in the repro studies than those used in the tox studies. Such difference was related to the age of animals. However, the sponser failed to explain why such age differences exist in the first place. TAK 375 also caused a significant decrease in mean wt and wt and food intake at 600mg/kg groups. There was an increase in the number of irregular estrous cycles in HDf relative to the control, melatonin is known to affect estrous cycle therefore, this effect *maybe* related to the pharmacological effects of the drug. A significant increase in mean pre- and post-implantation loss were seen in 60mg/kg but not in 600mg/kg/d group, significant decrease in # of implants and # of corpora lutea in 600mg/kg gr (# of implants was also reduced in 60mg/kg gr but not significantly), mean number of live embryos was reduced in 60&600mg/kg grs. None of these effects were seen in the 2nd mating of treated males to untreated females but, large interanimal variations was present. TAK375 had no effect on fertility or copulatory indices. Mean absolute and relative wt of the liver and adrenals of both males and females were significantly increased without any histopathology in 600mg/kg group. There were no drug effect on sperm count, motility, or morphology.

The NOEL in males was 60mg/kg (due to changes in organ wts at 600mg/kg), in females was 6mg/kg (due to irregular estrous and effects on # of corpora lutea, pre- and post-implantation loss, and # of live fetuses at 60mg/kg), and the NOEL of the drug on fertility and reproductive performance is 600mg/kg for the male and females.

The sponsor conducted another study using 20, 60, and 200mg/kg doses of TAK375 administered orally to pregnant rats and to male rats pre-mating (similar to the study above). Except for the increase in number of irregular estrous cycles, none of the other findings were observed in the current study at the 60mg/kg dose which is the common dose in both studies. The sponsor indicated that melatonin affects estrous and that these findings are only extensions of the drug's pharmacological effects. The NOAEL in males is 60mg/kg/d and 20mg/kg/d in females. The drug did not affect fertility parameters in males upto 200mg/kg dose. in females the NOAEL is <60mg/kg/d due to the effects on estrous cycle in this study and other reproductive parameters in the previous study.

In yet another study, the sponsor measured plasma hormone levels 1hr post the last dose: LH, FSH, T4, T3m ACTH, and corticosterone; LH & FSH levels were measured in ovariectomized females and testosterone and prolactin levels were determined only in males and ovariectomized females.

The results were highly variable in all groups: there was a significant decrease in testosterone levels and in T4 in the 200mg/kg male and female groups respectively. ACTH levels were decreased in all drug groups relative to the control, but were higher than the control in female rats where the control value was 0. In summary, the sponsor attributed the decrease in testosterone and T4 levels to the pharmacological effects of the drug being a melatonin agonist. Although this may be true, a drug adverse effect can not be ruled out since some of these changes were seen to correlate to some histopath and/or organ wt changes, or effects on reproductive parameters in previously conducted rat tox and reproductive studies.

Note: This reviewer (Adam Wasserman) considers the NOAEL dose in the fertility and early embryonic development studies to be 600 mg/kg/day for males and 20 mg/kg/day for females. The 60 mg/kg/day dose level produced in the initial and repeat Segment I studies irregular estrus cycles and in the initial study a reduction in the number of live embryos at dose levels of ≥ 60 mg/kg/day. Other alterations such as decreased pre- and post-implantation loss, decreased implants, and decreased corpora lutea were observed at either the 60 mg/kg/day or 600 mg/kg/day level only in the first study. Repeat of the study did not detect these latter changes at 60 mg/kg/day or 200 mg/kg/day though the estrus irregularity was present. As there is no explanation for the differences between the two studies (i.e. age of animals, treatment-duration of females, dose formulation, etc. were similar) the most conservative approach would be to define the 20 mg/kg/day level, at which dose no toxicity was observed as the appropriate NOAEL in these studies. Note that no toxicokinetic data are available from this study but a body surface area conversion for comparison to human exposure would predict a margin of exposure of 26-fold. It may be noted that the 6 mg/kg/day dose level for which toxicokinetic data was supplied in a separate study would provide a margin of exposure of 138-fold. No evaluation of TAK-375 metabolites was conducted and thus the exposure at the NOAEL dose to the active metabolite M-II cannot be judged.

Study title: A Dose-Finding Study for the Effects of TAK-375 on Embryo-Fetal Development in Rats

Key study findings:

- Rats treated with TAK-375 at doses of 0 (0.5 w/v% MC), 10, 100 or 1000 mg/kg/day demonstrated evidence of significant maternal toxicity including mortality with doses of 1000 mg/kg/day. This HD group also showed evidence of increased post-implantation loss and a decrease in live fetuses with decreased fetal weight. Evidence of external abnormalities (edema) is reported in 1/14 fetuses from the HD group but is not consistent with Sponsor's table which suggests $50.0 \pm 70.7\%$ edema in this group.

Study no.: M-11-00012.001

Volume #, and page #: N/A

Conducting laboratory and location: Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd., Yamaguchi 743, Japan

Date of study initiation: October 30, 1997

GLP compliance: No

QA reports: yes () no (X)

Drug, lot #, and % purity: TAK-375, Lot # M375-002. Purity (— %), stability, and homogeneity established by Sponsor and documented in subsequent study (M-11-0036) Appendix.

Methods

Doses: 0 (0.5 w/v% MC), 10, 100 or 1000 mg/kg/day Days 6-17

Species/strain: Sprague-Dawley rats

Number/sex/group: 6 dams/dose group

Route, formulation, volume, and infusion rate: Oral solution in 0.5 w/v% MC, 10 mL/kg/day

Satellite groups used for toxicokinetics: None

Study design:

Parameters and endpoints evaluated:

General signs once daily during non-treatment period and 3 times a day during treatment period (before and after dosing)

Body weight on days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation

Food consumption determined over a 24 hr period on Days 0, 6, 8, 10, 12, 14, 16, 18, and 19 of gestation

Necropsy conducted on Day 20 of gestation

Organ weights determined for liver and adrenal glands

Placenta observed for macroscopic abnormalities and weighed

Fetuses observed for mortality rate, number of live fetuses, sex ratio, fetal weight and gross morphologic abnormalities

Results

Mortality: No deaths were observed during the study in vehicle-treated rats or those in the TAK-375 10 and 100 mg/kg/day groups. Significant treatment-related mortality was observed in the TAK-375 1000 mg/kg/day dose group in which 4/6 animals died. This occurred typically after the first or second dose of the study. Necropsy of these animals did not reveal any gross findings which would provide an obvious explanation for the mortality.

Clinical signs: TAK-375-related clinical signs observed at 100 mg/kg/day were limited to salivation, while at 1000 mg/kg/day dams demonstrated reduced respiratory rate, lying on one side, lacrimation and mydriasis.

Body weight: Body weight gain was significantly reduced in the surviving 1000 mg/kg/day dams. No alteration in body weight was observed with dosing $\leq$ 100 mg/kg/day.

Food consumption: Paralleling body weight gain findings, food consumption was significantly decreased in surviving 1000 mg/kg/day group animals.

Toxicokinetics: Not done

Necropsy: Necropsy of the 2/6 dams surviving from 1000 mg/kg/day group revealed discoloration of the liver, enlarged adrenal glands and a small thymus. Increased liver and adrenal weight was found in these animals. No macroscopic findings were observed in animals administered TAK-375 $\leq$ 100 mg/kg/day nor were alterations in organ weight observed.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Examination of pregnant dams on Day 20 of gestation revealed no change in the number of corpora lutea but a slight and significant decrease in the number of implants observed on necropsy (0: 15.0; 10: 15.3; 100: 15.3; 1000 10.5*) which is not considered treatment-related as these alterations would occur prior to the start of dosing on Day 6 of gestation. Treatment-related effects were observed, however, with an **increase in post-implantation loss** with doses $\geq$ 100 mg/kg/day (0: $5.6 \pm 7.8\%$; 10: $5.6 \pm 7.4\%$; **100: $13.7 \pm 22.3\%$; 1000: $43.8 \pm 61.9\%$**). Fetal observations are described in the table below which is adapted from the Sponsor. An apparent large increase in post-implantation loss was observed in the 1000 mg/kg/day treatment group with a mild increase in the 100 mg/kg/day group. Statistics were not significant in this parameter and only 2 HD dams could be included in evaluations. The 1000 mg/kg/day group dams also appeared to have a slight decrease in live fetuses compared with controls and lower TAK-375 treatment groups. Body weight was observed to be significantly decreased in male and female fetuses from dams in the 1000 mg/kg/day treatment group. One of the two litters in surviving dams administered 1000 mg/kg/day had only one surviving fetus which demonstrated edema. The other litter in the dose group had no abnormalities. **Note: It is unclear how an external abnormality % analysis would generate $50\% \pm 70.7\%$ in this case.** No obvious external abnormalities were observed in animals treated with 100 mg/kg/day TAK-375 or lower. The placentas/amnions in each group were not affected by treatment with the exception of the placenta in the surviving fetus from 1 of 2 HD dams in which discoloration was observed. It is notable that all other fetuses in this dam appear to have been reabsorbed.

Fetal Observations				
Dose	0 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day
Observation				
Post-implantation loss (%)	5.6	5.6	13.7	43.8
Live fetuses (%)	14.2	14.5	13.5	7.0
Body weight (g)				
Males	3.62	3.67	3.31	2.21*
Females	3.36	3.39	3.07	1.88*
Sex ratio (% male)	47	47	40	35
External abnormalities (%)	0	0	0	50

Study title: Effects of TAK-375 on Embryo-Fetal Development in Rats

Key study findings:

- Administration of TAK-375 at doses of 0 (0.5 w/v% MC), 10, 40, 150 or 600 mg/kg/day over days 6-17 of gestation in pregnant rats produced dose-related maternal toxicity which in the 150 mg/kg/day group was characterized by clinical signs of salivation, a transient reduction in body weight for the first 3 days of the study and an increase in liver weight while dams in the 600 mg/kg/day group demonstrated ataxia, decreased spontaneous movement, lying on one side, reduction in feces and salivation. Body weight was reduced in this group compared with controls as was food consumption. On necropsy an enlarged adrenal was found in a single animal and as a group adrenal gland weight was increased (involving several animals when data was examined individually) as was liver weight.
- Examination of fetuses from litters of dams treated with TAK-375 or vehicle revealed that in 150 mg/kg/day litters a mild but non-significant reduction in fetal body weight in both sexes could be seen while malformations characterized as diaphragmatic hernia was noted. Variations in 150 mg/kg/day litters were primarily skeletal and consisted of irregularly shaped lumbar ribs and scapula which were within the numerical frequency of historical control data. Fetuses from 600 mg/kg/day litters demonstrated a statistically significant decrease in body weight in both sexes and malformations which included cysts on the external genitalia and diaphragmatic hernia. An observation of increased number of lumbar rib was noted with doses $\geq$ 150 mg/kg/day but was not statistically significant. Anatomical variations noted included irregularly shaped lumbar ribs and scapula as well as an observation of split sternbra.
- In agreement with the Sponsor, **the maternal NOAEL is considered to be 40 mg/kg/day TAK-375 and the embryofetal NOAEL is considered to be 40 mg/kg/day TAK-375 as well.**

Study no.: M-11-36

Volume #, and page #: N/A

Conducting laboratory and location: Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd., Yamaguchi 743-8502, Japan

Date of study initiation: March 13, 1998, Completed November 11, 1998

GLP compliance: Yes, Japan

QA reports: yes (X) no ()

Drug, lot #, and % purity: TAK-375, Lot # M375-002, purity $\geq$ 95%. Purity, homogeneity and stability documentation in Appendix on an analogous TAK-375 lot (M375-002).

Methods

Doses: 0 (0.5 w/v% methylcellulose), 10, 40, 150 and 600 mg/kg/day on Days 6-17 of gestation

Species/strain: Female Sprague-Dawley Rats (J:CD(SD)IGS); females weighed 225-288 g at study randomization;

Age: 12-14 weeks at initiation of dosing.

Number/sex/group: 16-20 dams/group

Route, formulation, volume, and infusion rate: Oral gavage, TAK-375 dissolved in 0.5 w/v% MC and administered as 10 mL/kg/day

Satellite groups used for toxicokinetics: None

Study design: (from Sponsor's submission)

Group	Test article	Dosage level (mg/kg/day)	Dosage volume (mL/kg)	Concentration of dosing suspension (w/v%)	Number of pregnant animals
1	Control (0.5 w/v% methylcellulose solution)	0	10	0	20
2	TAK-375	10	10	0.1	20
3	TAK-375	40	10	0.4	20
4	TAK-375	150	10	1.5	20
5	TAK-375	600	10	6.0	16

Parameters and endpoints evaluated:

- Clinical signs: Observed once daily except during treatment period in which clinical signs were observed three times daily (pre-dose, immediately post-dose, and 0.5 – 2 hr after dosing).
- Body weight: Dams weighed on Days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation
- Food consumption: Individual 24-hr food consumption was determined on Days 0, 6, 8, 10, 12, 14, 16, 18 and 19 of gestation
- Necropsy: Conducted on day 20 of gestation. Dams were sacrificed by exanguination under CO₂. The uterus, ovaries, and main organs were observed macroscopically. Organs weighed included the liver, adrenal glands and the carcass. The liver and adrenals were fixed. Observations used for fertility measures included the number of corpora lutea, number of implantations, number of live/dead embryos and fetuses. The number of resorptions, placental remnants and macerated fetuses were recorded. The placenta, amniotic fluid and amnion were observed macroscopically. The sex was determined for live fetuses and they were then scored for external abnormalities and weighed. Half the live fetuses in each litter were eviscerated and fixed and dehydrated in 99 v/v% ethanol, stained with alizarin red S and examined for skeletal abnormalities. The degree of ossification was examined in the sacro-caudal vertebra. The remaining half of fetuses in each litter were fixed in 10 w/v% formalin and examined for visceral abnormalities. Tissues with gross lesions were preserved for possible future evaluation and in this case the same tissues from 3 control fetuses were examined.

Statistical evaluation of the data: From Sponsor's submission (verbatim):

The data on maternal body weight, maternal food consumption, numbers of corpora lutea, implants, dead embryos/fetuses (resorptions, placental remnants, macerated fetuses), live fetuses and sex ratio, fetal weight by sex and the number of ossified sacro-caudal vertebrae in fetuses were analyzed generally on a litter basis [6] and statistically as follows. First, the data were tested by Bartlett's test [7] for homogeneity of variance. When the variances were homogeneous, the Dunnett test [8] was performed to compare the mean in the control group with that in each dosage group. When the variances were heterogeneous, a Dunnett type test [9, 10] which is based on ranked data was performed to compare the mean rank in the control group with that in each dosage group.

The data on frequency of pre- or post-implantation loss, external, visceral or skeletal abnormalities (variations) or each external, visceral or skeletal abnormality (variation) in the fetuses and abnormal placentae were statistically analyzed as follows. When the results indicated a significant difference among the groups, the mean ranks of the dosing groups were compared with that of the control group utilizing a Dunnett type

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test [9, 10].

Bartlett's test was conducted at the significance level of 0.05, and the other tests were conducted at the two-tailed significance levels of 0.05 and 0.01. The Dunnett (type) test was performed using the SAS function PROBMCM [11].

Results

Mortality: A single death was noted at the 600 mg/kg/day level. This dam died on the 3rd dose (Day 8). The Sponsor noted lying on one side and bradypnea, hypothermia, absence of feces, lacrimation and stained fur around the nose after each dose.

Clinical signs: No abnormal clinical signs were observed at ≤ 40 mg/kg/day. Salivation was noted with the 150 mg/kg/day dose while dams given 600 mg/kg/day demonstrated a reduction in spontaneous activity, ataxia, reduced feces production, lying on one side and salivation.

Body weight: Body weight was unchanged in dams treated with ≤ 40 mg/kg/day TAK-375. A transient decrease in body weight gain was observed in rats treated at the 150 mg/kg/day level for the first 3 days of the study. Dams in the 600 mg/kg/day group demonstrated a decrease in body weight gain over the entire treatment period (days 6-18) and body weight was depressed relative to control animals on days 10, 12, 14, 16, 18 and 20 of gestation.

Food consumption: Food consumption was not affected at dose levels ≤ 150 mg/kg/day. A decrease in food consumption was observed in the 600 mg/kg/day group when compared with controls on all study days at which this was determined except for Day 18 and 19 of gestation.

Toxicokinetics: This was completed in a separate study M-11-00173.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): The single dam in the 600 mg/kg/day level found dead demonstrated pulmonary congestion, enlargement of the adrenal glands, an accessory spleen and petechiae in the stomach. Other than an enlarged adrenal gland in another dam in the 600 mg/kg/day group, no other animals in this group or in any treatment group showed any macroscopic alterations that appeared to be treatment-related. Organ weights were unchanged at dose levels ≤ 40 mg/kg/day. Liver weight increased with dose levels ≥ 150 mg/kg/day and adrenal gland weights were increased at the 600 mg/kg/day dose level. The weight of the carcass was decreased at the highest dose level as well. The Sponsor reports there were no differences in treated groups when compared with controls on the number of corpora lutea or implantations. While true, there is an unaccountable difference in pregnancy rates between the groups which remains unexplained by the Sponsor.

	Treatment Group				
	0	15	40	150	600
# Females <u>not</u> pregnant (%)	0/20	0/20	0/20	0/20	4/20 (20%)

According to study methodology, all females were observed to have a vaginal plug prior to randomization to treatment group which was based on body weight.

Post-implantation loss appeared to be increased at the 600 mg/kg/day level when compared with controls but this did not reach statistical significance due to the high variability of the finding ($12.2 \pm 26.2\%$ vs. $2.4 \pm 5.1\%$). The number of live fetuses tended to be depressed in the 600 mg/kg/day group as well but again this was not statistically significant and this group showed greater variability than others, including the control group litters. Body weight was significantly decreased in the 600 mg/kg/day group litters ($\downarrow 18\%$ ♂, $\downarrow 19\%$ ♀) compared with controls and may have been slightly suppressed in the 150 mg/kg/day group litters but the difference was not statistically significant at this dose level.

	Maternal TAK-375 dose (mg/kg/day)				
	0	10	40	150	600
Post-implantation loss (%)	2.4 ± 5.1	3.1 ± 4.6	3.5 ± 4.9	4.2 ± 5.7	12.2 ± 26.2
Live fetuses	14.2 ± 1.8	14.9 ± 1.1	14.4 ± 2.0	14.3 ± 1.9	13.0 ± 4.0
Body weight (g)					
Male	3.50 ± 0.24	3.45 ± 0.17	3.46 ± 0.21	3.38 ± 0.28	$2.87 \pm 0.33^{**}$
Female	3.33 ± 0.20	3.27 ± 0.18	3.23 ± 0.27	3.22 ± 0.28	$2.71 \pm 0.33^{**}$

Offspring (malformations, variations, etc.):

Fetuses were examined for abnormalities according to the methods described previously. A listing of the observed malformations and variations in fetuses of treated and control dams is presented below.

Note that this reviewer considered the following factors in determining the potential significance of the findings:

1. Malformation/variation should exhibit dose-dependent increase in incidence
2. Malformation/variation should be statistically significant different from control
3. Malformations observed at a dose level that is not statistically significant but appears increased above control and achieves statistical significance at a higher dose level.
4. Malformations should be observed in more than one litter of a treatment group; or,
5. If malformations were observed in only one litter, incidence should surpass the historical control data

Observed Fetal Malformations and Variations

	Maternal TAK-375 dose (mg/kg/day)				
	0	10	40	150	600
External Findings					
# fetuses examined	284	298	287	286	195 [†]
% malformations (total)	0.4 ± 1.9	0.6 ± 2.0	0	0	2.1 ± 4.4
Omphocele	0	0	0	0	0.6 (1)
Cyst, external genitalia	0	0	0	0	1.5 (1)*
Visceral Findings					
# fetuses examined	155	160	155	154	107 [†]
% malformations (total)	0	0	0.7 ± 3.2	1.8 ± 4.4	$12.8 \pm$

					20.6
Aortic arch absence	0	0	0.7 (1)	0	0.9 (1)
Diaphragmatic hernia	0	0	0	1.2 (2)	11.9 (6)**
% variations (total)	0	1.7 ± 4.2	0.6 ± 2.8	3.2 ± 7.1	2.0 ± 5.2
Dilation of renal pelvis	0	0.6 (1)	0.6 (1)	0	2.0 (2)
Skeletal Findings					
# fetuses examined	129	138	132	132	88 [†]
% malformations (total)	1.0 ± 4.5	0	0	0.7 ± 3.2	2.3 ± 6.1
↑ number of lumbar vert.	0	0	0	0.7 (1)	2.3 (2)
% variations (total)	12.1 ± 15.3	12.8 ± 19.2	19.5 ± 17.5	23.3 ± 19.7	51.8 ± 29.4 **
Lumbar rib	7.7 (7)	9.3 (7)	15.6 (13)	14.8 (12)	24.9 (10)
Split sternebra	0	0	0	0	1.4 (1)
Irregularly shaped scapula	0	0.6 (1)	0.8 (1)	6.1 (5)	29.8 (9)**
Note: Only fetal abnormalities that appear to be dose-dependent are specifically listed in table (), Numbers in parenthesis indicate the number of litters affected [†], only 14 litters examined *, significantly different from control animals, p ≤ 0.05 **, significantly different from control animals, p ≤ 0.01					

Potential treatment-related fetal malformations observed with external evaluation included cysts on the external genitalia. Though statistically significant this was observed only in 3 fetuses in the litter of a single dam treated with TAK-375 600 mg/kg/day. Visceral examination, however, revealed a clear and statistically significant dose-dependent development of diaphragmatic hernia in fetuses of dams in the 600 mg/kg/day group. This finding was observed in 6 litters so this was not isolated to a single dam. This malformation was observed in two 150 mg/kg/day litters but the increase was not statistically significant over control values. Skeletal malformations which appeared to exhibit dose-dependence included an increase in lumbar vertebra number which was not found in fetuses of dams in the ≤ 40 mg/kg/day group but was found in those of dams in the 150 and 600 mg/kg/day groups. Fetal abnormalities which were considered variations and which may be treatment-related included dilation of the renal pelvis which was found in 2 animals in the 600 mg/kg/day group from two different litters while only 1 animal was found at the 15 and 40 mg/kg/day group litters. Irregularly shaped lumbar rib was more common with higher dose group litters but was frequently observed in the control litters as well. The increased frequency of observation of irregularly shaped scapula, however, was dose-dependent and statistically significant. This finding was not observed in control group litters while ~ 30% of fetuses from 600 mg/kg/day group litters had this variation. A split sternebra was found in 2 fetuses in the 600 mg/kg/day group litters and was not found in any of the lower treatment group litters.

No unusual findings were observed when the placenta or amnia of treated or control dams were examined.

The results of this study indicate a NOAEL (maternal toxicity) for TAK-375 is considered to be 40 mg/kg/day due to clinical signs, reduced body weight and alterations in organ weight, principally liver and adrenal glands, with dosing above this level. The NOAEL (embryofetal

toxicity) is also considered to be 40 mg/kg/day due to a trend toward increased incidence of diaphragmatic hernia and increased lumbar vertebra number associated with fetuses of dams in the 150 mg/kg/day treated group, which although not statistically significant, appear to be the leading edge of a dose-dependent increase. Additional adverse effects, though not statistically significant, was the trend towards reduced body weight in 150 mg/kg/day litters which achieved significance at the highest exposure.

Study title: Plasma Concentrations of TAK-375 and its Metabolite M-II (2S, 8S), in Pregnant Rats Treated Orally with TAK-375

Key study findings:

- Pregnant 13-week old Crj:CD(SD)IGS rats analogous to those used in the Segment II reproductive toxicology study described above were administered TAK-375 at doses of 40 or 150 mg/kg/day for gestation days 6-17 to determine the toxicokinetic parameters of TAK-375 and M-II (both 2S, 8S [active] and 2R, 8S metabolites).
- At the NOAEL dose determined in the previous embryofetal development study, 40 mg/kg/day, TAK-375 AUC_{0-24h} exposure was 17,790 ng•h/mL while M-II (2S, 8S) exposure was 10,772 ng•h/mL.
- When NOAEL exposure values are compared to human values estimated from studies conducted in humans, the margin of exposure at the NOAEL dose provides for a 1,892-fold margin for TAK-375 while for M-II (2S, 8S) this margin is 45-fold.

Study no.: M-11-173

Volume #, and page #: N/A

Conducting laboratory and location: Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd., Osaka, 532-8686, Japan

Date of study initiation: 6/14/1999

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: TAK-375, Lot # M375-013, purity — %. Purity, homogeneity and stability documentation in Appendix on an analogous TAK-375 lot (M375-002).

Methods

Doses: TAK-375 at 40 or 150 mg/kg/day for gestation days 6-17.

Species/strain: — CD(SD)IGS rats (female weight: 240-293 g at initiation of dosing)

Number/sex/group: 4 pregnant females/dose level; 3 dams/dose level used for TK analysis

Route, formulation, volume, and infusion rate: Oral gavage in 0.5 w/v% MC, 10 mL/kg

Satellite groups used for toxicokinetics: See above.

Study design:

Group	Test article	Dosage level (mg/kg/day)	Dosage volume (mL/kg)	Concentration of dosing suspension (w/v%)	Number of animals		
					Mated	Pregnant	Examined
1	TAK-375	40	10	0.4	4	4	3
2	TAK-375	150	10	1.5	4	4	3

Parameters and endpoints evaluated:

General signs: Once daily during non-treatment period and twice daily (pre-dose and 0.5 – 2 hr post-dose) during treatment period.

Body weight: Dams weighed on days 0, 6, 8, 10, 12, 14, 16, and 18 of gestation.

Food consumption: 24-hour food consumption value obtained on days 6, 8, 10, 12, 14, and 16 of gestation.

Necropsy: Animals were sacrificed on Day 18 of gestation with exanguination under ether anesthesia. Visceral organs were examined microscopically and implantation sites in uterus were noted.

Toxicokinetics: Plasma concentration of TAK-375 and the metabolites M-II (2S, 8S) and (2R, 8S) were determined from plasma samples (0.25 mL) obtained from tail vein on Day 6, 10 and 17 of dosing. Time points evaluated included a 0.25, 0.5, 1, 2, 4, 8 and 24 hr after dosing as well as pre-dose values on Days 10 and 17.

Results

No mortality was observed in the 40 or 150 mg/kg/day dose groups. No clinical signs, body weight or food consumption changes were noted. Necroscopic examination of dams on Day 18 did not reveal any abnormality.

Toxicokinetics:

The Sponsor's table is reproduced below.

Toxicokinetic measurements of TAK-375, M-II (2S, 8S [T-163302]) and M-II (2R, 8S [T-163306])

Dose (mg/kg/day)	Analyte	No. of dosing	Female (N=3)			
			Tmax (h)	Cmax (ng/mL)	AUC _{0-24h} (ng·h/mL)	
40	TAK-375	1st	1.0 (0)	6996 (151)	35819 (9704)	
		5th	1.3 (0.6)	3210 (1509)	13077 (4813)	
		12th	1.0 (0)	3305 (857)	17790 (4286)	
	T-163302	1st	2.0 (0)	1502 (158)	15048 (2449)	
		5th	2.0 (0)	1554 (93)	9787 (888)	
		12th	2.0 (0)	1138 (91)	10772 (865)	
	T-163306	1st	2.7 (1.2)	567 (96)	5897 (813)	
		5th	2.0 (0)	582 (68)	3517 (272)	
		12th	2.0 (0)	478 (45)	3719 (261)	
	150	TAK-375	1st	1.0 (0)	20765 (4981)	161852 (18389)
			5th	0.8 (0.3)	8454 (2060)	63363 (20725)
			12th	0.8 (0.3)	8011 (815)	81595 (22633)
T-163302		1st	4.0 (3.5)	1546 (255)	22648 (7750)	
		5th	1.3 (0.6)	1637 (46)	14786 (1731)	
		12th	1.3 (0.6)	1766 (76)	17373 (2827)	
T-163306		1st	3.3 (2.3)	696 (109)	10130 (1159)	
		5th	1.7 (0.6)	738 (64)	6977 (1139)	
		12th	1.3 (0.6)	715 (46)	7489 (990)	

Mean (S.D.).

Generally, exposure to TAK-375 and metabolites decreases with repeated exposure (though the M-II (2R, 8S) is relatively stable). Increases in TAK-375 dose produced a less than dose-proportional effect on C_{max} but a slightly greater than dose-proportional effect on AUC_{0-24} when examining TAK-375 as the anlylate. When analyzing the M-II (2S, 8S) epimer which is known to be pharmacologically active, C_{max} remains relatively flat with repeated exposure but AUC_{0-24} decreases slightly. Accumulation does not take place with repeated dosing for TAK-375 or the monitored metabolites. Toxicokinetic parameters obtained from dams on Day 12 reveal the following values:

Day 12 Toxicokinetic Parameters			
Dose	Anlylate	C_{max} (ng/mL)	AUC_{0-24} (ng•h/mL)
40 mg/kg/day	TAK-375	3,305	17,790
	M-II (2S, 8S)	1,138	10,772
	M-II (2R, 8S)	428	3,719
150 mg/kg/day	TAK-375	8,011	81,595
	M-II (2S, 8S)	1,766	17,373
	M-II (2R, 8S)	715	7,849

As can be seen in the table below, the NOAEL dose of TAK-375 in the rat embryofetal toxicology study provides a large safety margin for TAK-375 over the expected human clinical exposure. Although the M-II metabolite has a much lower safety margin at the NOAEL dose, this still provides a 21 – 47-fold margin for human use.

Safety Margins over Expected Human Clinical Exposure to 8 mg daily TAK-375 [‡]				
Dose (mg/kg/day)	Analyte			
	TAK-375		M-II (2S, 8S)	
	C_{max}	AUC	C_{max}	AUC
40 [NOAEL]	612-fold	1892-fold	18-fold	45-fold
150	1483-fold	8680-fold	28-fold	72-fold

[‡], Derived from Clinical Study in adults and elderly (01-01-TL-375-003) in which 16 mg Ramelteon was administered to subjects. Exposure for the 8 mg intended dose of Ramelteon is expected to be linear over the dose-range and therefore the Sponsor's estimate of C_{max} and AUC_{0-24} at 8 mg is: TAK-375 (5.8 ng/mL, 9.4 ng•h/mL), M-II (62.5, 241.3), M-I (4.3, 8), M-III (1.4, 3.5), M-IV (7 ng/mL, 57 ng•h/mL)

Study title: Effects of Intravenously Injected TAK-375 M-II (2S, 8S) on Embryo-Fetal Development in Rats

Key study findings:

- Intravenous administration of M-II (2S, 8S) to pregnant rats on Days 6-17 of gestation at doses of 0 (30 vol% PEG-400 in dH₂O), 10 or 30 mg/kg/day did not produce maternal toxicity at the highest dose level given. No effects of M-II were observed when assessing fetuses when considering body weight, sex ratio, %alive on cesarean section, or external, visceral or skeletal abnormalities when compared with fetuses from

control litters. Both **maternal and embryo-fetal NOAEL was considered by Sponsor and this reviewer to be 30 mg/kg/day M-II**. This reviewer is in cautious agreement despite to the finding of a single malformation, abnormal subclavian artery, in a single offspring of the 30 mg/kg/day litters (1.0% of fetuses; 1 of 17 litters with this abnormality) which is very uncommon based on a search of historical control data (0.04% fetuses; 4 of 1,460 of litters) of Charles River CR(SD)IGS rats between 1995 and 2000. It is possible this is a random malformation.

- The M-II (2S, 8S) maternal plasma C_{5min} obtained with the NOAEL dose of 30 mg/kg/day was 19,575 ng/mL on the 12th day of dosing (313-fold expected human C_{max} with therapeutic dosing). Limited conversion of the (2S, 8S) epimers to the (2R, 8S) epimers was found but did not achieve greater than 1.5% of the (2S, 8S) concentration at any time-point.

Study no.: M-11-187

Volume #, and page #: N/A

Conducting laboratory and location: Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd., Osaka, Japan

Date of study initiation: 8/6/1999

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: TAK-375 M-II (2S, 8S), Lot # M375-M201, purity — % as documented in Appendix. Stability confirmed during the experimental period as documented in Appendix.

Methods

Doses: 0 (30 vol% PEG-400 in dH₂O), 10 or 30 mg/kg/day TAK-375 M-II (2S, 8S)

Dose rationale: Doses chosen were determined from a preliminary dose range-finding study (M-11-168) in which no maternal or embryo-fetal toxicity was observed when 30 mg/kg/day was given i.v. to pregnant rats over gestation days 6-17. No visceral or skeletal examinations of fetuses were conducted, however. Justification for the high dose to be tested in the main study rests on the very high maternal plasma concentrations of M-II (2S, 8S) achieved with this maternal dose.

Note this study was not formally reviewed.

Species/strain: — :CD(SD)IGS rats

Number/sex/group: 18 dams/dose level

Wt: 235-298 g at time of randomization

Route, formulation, volume, and infusion rate: Intravenous administration of test article in 30 vol% PEG-400, 10 mL/kg per infusion

Satellite groups used for toxicokinetics: None, TK established from main study animals.

Study design:

Group	Test article	Dosage level (mg/kg)	Dosage volume (mL/kg)	Concentration of dosing solution (w/v%)	Number of animals	
					Mated	Pregnant ^{a)}
1	Vehicle	0	10	0	18	16
2	TAK-375 M-II (2S,8S)	10	10	0.1	18	17
3	TAK-375 M-II (2S,8S)	30	10	0.3	18	17

a): Three animals, 01F13, 02F10 and 03F11, died (accidental death), and one animal, 01F08, was not pregnant.

Vehicle : 30 vol% PEG 400

Parameters and endpoints evaluated:

General sings: Once daily during non-treatment period, twice daily during treatment period (once pre-dose, once 0.5 – 2 hr after administration).

Body weight: Weight determined on Days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation

Food consumption: 24 hr food consumption value determined on days 0, 6, 8, 10, 12, 14, 16, 18, and 19 of gestation.

Plasma concentration: Plasma samples were analyzed using $\square$ for the presence of both M-II (2S, 8S) and M-II (2R, 8S), the latter to determine potential interconversion, in 3 dams per treated group on Day 6 (1st dose) and Day 17th (12th dose) of gestation at 5 and 15 minutes as well as 4 hr after i.v. M-II (2S, 8S) administration.

Necropsy: Dams were killed on Day 20 of gestation and the ovaries, uterus and main internal organs were examined macroscopically for abnormality. The numbers of corpora lutea as well as implantation sites, resorptions, placental remnants, dead embryos/fetuses and live fetuses were determined. Placentas, amniotic fluid and amnions were examined for abnormality and the placenta was weighed. All live fetuses were examined externally for abnormalities and approximately half were fixed in formalin for visceral organ examination and the remaining fetuses were eviscerated and dehydrated in ethyl alcohol followed by staining with alizarin red S for skeletal examination. Visceral and skeletal examinations were only carried out on fetuses from the control and 30 mg/kg/day M-II group.

Results

Mortality (dams): No deaths were recorded in this study.

Clinical signs (dams): No treatment-related clinical signs reported.

Body weight (dams): There were no treatment-related effects on body weight.

Food consumption (dams): Food consumption was increased transiently during dosing period in dams given 10 mg/kg/day. Food consumption was increased throughout the dosing period in dams in the 30 mg/kg/day level. No effects on body weight were observed, however.

Toxicokinetics: M-II (2S, 8S) [T-163302] and M-II (2R, 8S) concentrations in plasma after intravenous administration of the M-II (2S, 8S) epimers are described in Sponsor's table below which was reproduced from the submission.

Table 3 Mean concentrations of T-163302 and T-163306 in female rat plasma

Dose (mg/kg/day)	Analyte		Concentration (ng/mL)			
			Pre	5 min	15 min	4 h
10	T-163302	1st	-	14437 (3492)	11109 (1215)	35 (10)
		12th	0 (0)	13633 (8564)	7173 (1985)	55 (10)
	T-163306	1st	-	14 (12)	32 (19)	0 (0)
		12th	0 (0)	13 (4)	33 (3)	0 (0)
30	T-163302	1st	-	26046 (1506)	28111 (2164)	137 (88)
		12th	0 (0)	19575 (1862)	22806 (1473)	132 (61)
	T-163306	1st	-	69 (8)	118 (7)	2 (3)
		12th	0 (0)	57 (14)	103 (8)	2 (3)

Mean (S.D.); n=3.

-: No sample.

Values less than the quantitation limit (0 ng/mL) were presumed to be 0.

As can be seen in the above table, non-negligible levels of the epimers M-II (2R, 8S) were found to be circulating in the plasma but was less than 1.5% of M-II (2S, 8S) levels at all time-points. Some reduction in C_{max} was observed with repeated dosing at both levels of metabolite dosing although a slight increase in plasma concentrations was noted with repeated dosing at the 4 hr time-point. M-II C_{max} increased slightly less than dose-proportionally with increasing dose.

Terminal and necropsic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): No abnormalities were found in dams treated with either dose of M-II as compared with control animals. Examination of placenta, amnion and amniotic fluid did not reveal any treatment-related effects. There were no differences between treatment groups on numbers of corpora lutea, implantations or the % post-implantation loss. A slight decrease in the number of live fetuses in the 30 mg/kg/day M-II group did not reach the level of statistical significance when compared with control (13.4 ± 2.5 live fetuses vs. 14.6 ± 1.3 , respectively). Fetal body weight and sex ratio were unaffected by treatment with M-II.

Offspring (malformations, variations, etc.):

As can be seen in the table below, there were no significant increases in external, visceral, or skeletal malformations or variations with intravenous administration of up to 30 mg/kg/day M-II (2S, 8S) metabolite. A single incidence of abnormal subclavian artery origin observed in the 30 mg/kg/day group was found in a single animal in a single litter. The historical incidence in animals of this strain from the supplier and lab was not supplied by the Sponsor. A search of the Historical Control Database: A Joint Project of MARTA and MTA online for reproductive toxicology studies conducted with CD(SD)IGS rats from Charles River from 1995 – 2000 using the intravenous route picked up 10 studies with 239 total evaluable litters consisting of 1,601 total fetuses evaluated. The search term used was "malposition subclavian artery branch". No visceral abnormality of this type was found in these studies. When the search included all routes of administration and vehicle types in CD(SD)IGS rats from Charles River between 1995 – 2000, 63 studies were picked up with 1,460 evaluable litters consisting of 10,062 fetuses. Of these fetuses, 0.04% (a single fetus in 4 litters) exhibited a "malpositioned subclavian artery branch".

	Maternal M-II (2S, 8S) dose (mg/kg/day i.v.)		
	0	10	30
External Findings			
# fetuses examined	233	245	228
% malformations (total)	0	0	0
Visceral Findings			
# fetuses examined	122	Not examined	122
% malformations (total)	0		1.0 ± 4.0
Abnormal subclavian artery origin	0		1.0 (1)
% variations (total)	0		2.5 ± 5.5
Left umbilical artery	0		1.5 (2)
Dilation of renal pelvis	0		1.0 (1)
Skeletal Findings			
# fetuses examined	110	Not examined	106
% malformations (total)	0		0
% variations (total)	18.9 ± 19.9		18.1 ± 22.3
Dumb-bell shaped thoracic vt., body	1.9 (2)		2.0 (1)
Irregular lumbar rib	14.2 (6)		16.1 (8)
Note: Only fetal abnormalities that appear to be dose-dependent are specifically listed in table (), Numbers in parenthesis indicate the number of litters affected All malformations and variations $p > 0.05$ (30 mg/kg/day vs. control)			

The biological significance of the single incidence of abnormal subclavian artery origin in a fetus of a 30 mg/kg/day litter is difficult to interpret. The Sponsor considers the NOAEL for both maternal toxicity and embryofetal toxicity to be 30 mg/kg/day. This reviewer cautiously agrees with that assessment.

Study title: Range-Finding Study for the Effects of TAK-375 on Embryo-Fetal Development in Rabbits

Key study findings:

- Mated female $\approx$ JW rabbits, age 16-weeks, were administered TAK-375 (or 0.5 w/v MC) at doses of 0, 30, 100, 300 or 1000 mg/kg/day on gestation days 6-18 with sacrifice and necropsy conducted on Day 28 to examine the appropriate dose range for a subsequent Segment II embryo-fetal toxicity study in rabbits.
- All female rabbits in the 1000 mg/kg/day dose level died during the treatment period. Clinical signs and ↓ food consumption without effect on body weight was noted in dams receiving 300 mg/kg/day but all other measured maternal and fetal parameters were unaffected by this dose of TAK-375.
- Although NOAELs were not proposed by the Sponsor, this reviewer considers the NOAEL for maternal toxicity to be 30 mg/kg/day while the apparent embryofetal NOAEL is 300 mg/kg/day.

Study no.: M-11-00612

Volume #, and page #: N/A

Conducting laboratory and location: Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd. Yamaguchi, Japan

Date of study initiation: 3/16/1998

GLP compliance: No

QA reports: yes () no (X)

Drug, lot #, and % purity: TAK-375, lot # M375-004; purity not recorded. No stability information.

Methods

Doses: TAK-375 0 (0.5 w/v% MC), 30, 100, 300 or 1000 mg/kg/day days 6-18 of gestation

Species/strain: — :JW rabbits ♂ }
♀ }

Number/sex/group: 6 mated females/group

Route, formulation, volume, and infusion rate: Oral gavage, test article dissolved in 0.5 w/v% MC and administered as 10 mL/kg

Satellite groups used for toxicokinetics: Not done

Study design:

Parameters and endpoints evaluated:

General signs: Once daily during non-treatment period and three times daily during treatment period (pre-dose, 30-45 min post-dose and 1-2 hr post-dose).

Body weight: Obtained on days 0, 6, 8, 10, 12, 14, 16, 18, 19, 22, 25 and 28 of gestation

Food consumption: Obtained on days 0, 6, 8, 10, 12, 14, 16, 18, 19, 22, 25 and 27 of gestation

Necropsy: Conducted on Day 28 of gestation. Organ weights recorded included liver and adrenal glands only. Placenta and amnion were examined macroscopically for abnormalities and the placenta was weighed.

Fetuses: Fetal mortality, number of live fetuses, sex ratio, fetal weight and external characteristics of live fetuses were recorded

Results

All female rabbits receiving 1000 mg/kg/day TAK-375 died during the dosing period. Clinical signs observed included lying on one side, creeping, convulsions, rigidity, bradypnea, dyspnea and myosis. No other dose level was associated with mortality. Clinical signs associated with the 300 mg/kg/day group included lying on one side, creeping, decreased spontaneous activity and myosis with later development of decreased feces. Maternal body weight was not affected with dosing $\leq$ 300 mg/kg/day although food consumption was noted to be decreased significantly compared with control animals at dose groups of $\geq$ 100 mg/kg/day. Necropsy of all groups did not reveal treatment-related abnormalities or organ weight changes when compared with controls. The number of corpora lutea and implantations was not different in dams receiving $\leq$ 300 mg/kg/day TAK-375. The placenta and amnion of animals in all groups with doses $\leq$ 300 mg/kg/day were unremarkable. No adverse fetal effects were noted with

external examination, fetal weight, sex ratio, mortality and number of live fetuses in the litters of dams receiving ≤ 300 mg/kg/day TAK-375.

2003 03 27 10:00 AM

Study title: Effects of TAK-375 on Embryo-Fetal Development in Rabbits

Key study findings:

- Administration of TAK-375 at doses of 0 (0.5 w/v% MC), 12, 60, or 300 mg/kg/day over days 6-18 of gestation in pregnant σ :JW rabbits produced no adverse effects with dose levels ≤ 60 mg/kg/day. Administration of 300 mg/kg/day to dams was associated with the development of clinical signs of toxicity and a transient reduction of body weight gain over the first third of the dosing period. No alterations in corpora lutea or implantation sites were noted at any dose level.
- Examination of fetuses from litters of dams treated with TAK-375 or vehicle no differences in the number of live fetuses, body weight, or the sex ratio. External, visceral and skeletal examination of the live fetuses did not reveal a treatment-related increase in malformations or variations and the degree of ossification of the sacro-caudal vertebra were not different between the 300 mg/kg/day litters and the control litters. An apparent increase in the incidence of lumbar rib observation was noted in the 300 mg/kg/day litters compared with controls but the incidence was within the historical range of the laboratory with this strain and species.
- In agreement with the Sponsor, **the maternal NOAEL is considered to be 60 mg/kg/day TAK-375** (maternal $AUC_{0-24} = 22,000$ ng•h/mL, 2,340-fold expected human exposure) due to clinical signs of toxicity and transient reduction in body weight while **the embryofetal NOAEL is considered to be 300 mg/kg/day TAK-375** due to the absence of evidence of treatment-related effects on fetal survival, body weight, sex ratio, and external, visceral and skeletal anatomy. The embryofetal NOAEL in this study is associated with a maternal TAK-375 plasma exposure of 111,500 ng•h/mL which is approximately 462-fold above human AUC.

Study no.: M-11-00103.001

Volume #, and page #: N/A

Conducting laboratory and location: Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd., Yamaguchi, Japan

Date of study initiation: 6/4/1998

GLP compliance: Yes, Japan

QA reports: yes (X) no ()

Drug, lot #, and % purity: TAK-375, lot #M375-005, purity $\geq 95\%$, documented in appendix. Stability of the test article was confirmed by analysis after the completion of dosing and is documented in the appendix.

Methods

Doses: TAK-375 0 (0.5 w/v% MC), 12, 60 or 300 mg/kg/day for gestation days 6-18

Species/strain: σ :JW rabbits σ

Age: 16-18 weeks (σ); Wt: 2.84 – 3.74 kg at randomization

Number/sex/group: 20 mated females, 18-20 dams/group

Route, formulation, volume, and infusion rate: Oral gavage of test article in 0.5 w/v% MC given as 10 mL/kg

Satellite groups used for toxicokinetics: None. Main animals used for toxicokinetic analysis. See study #M-11-00188 for additional assessment of TAK-375 and metabolites.

Study design: (from Sponsor's submission)

Group	Test article	Dosage level (mg/kg/day)	Dosage volume (mL/kg)	Concentration of dosing suspension (w/v%)	Number of animals	
					Mated	Pregnant
1	Control	0	10	0	20	19
2	TAK-375	12	10	0.12	20	19
3	TAK-375	60	10	0.6	20	20 (1)
4	TAK-375	300	10	3	20	18 (2)

One dam in the control (01F17), 1 dam in the 12 mg/kg group (02F11) and 2 dams in the 300 mg/kg group (04F10 and 04F11) were not pregnant.

(): Number of abortions (03F09, 04F04, 04F06)

Control : 0.5 w/v% methylcellulose solution

Parameters and endpoints evaluated:

Clinical signs: Mortality and clinical signs assessed once daily during non-treatment period and 3 times daily during treatment period (before, 30-45 min after dosing and 1-2 hr after dosing).

Body weight: Assessed on gestation days 0, 6, 8, 10, 12, 14, 16, 18, 19, 22, 25 and 28

Food consumption: 24-hr value determined on gestation days 0, 6, 8, 10, 12, 14, 16, 18, 19, 22, 25 and 27

Toxicokinetics: Blood (0.5 mL) taken from auricular vein of 4 animals/group at 0.25, 0.5, 1, 3, 6, and 24 hr after dosing on Days 6 and 18 of gestation.

The concentration of TAK-375 was determined in 3 animals/group, however, at each time-point.

Necropsy: Conducted on Day 28 of gestation. Dams were killed by Nembutal euthanasia solution with exsanguination. Macroscopic observation of main internal organs including the uterus and ovaries was conducted. The liver and adrenal glands were the only organs weighed and fixed. Tissues with gross lesions were fixed and retained. Numbers of corpora lutea, numbers of implantations, dead embryos/fetuses (resorptions, placental remnants and dead fetuses) and live fetuses were counted. Placenta, amniotic fluid and amnions were examined macroscopically. Live fetuses were characterized as to sex to obtain a sex ratio, body weight, external abnormalities and visceral malformations and variations with microscopic examination of heart and kidneys. Skeletal examination was conducted after evisceration and fixation in 95 vol% ethanol followed by alizarin red S stain and malformations and variations noted.

Statistical evaluation of the data: From Sponsor's submission (verbatim):

Fisher's exact test [3] was performed for the frequency of abortion in order to compare the value in the control group with that in each dosage group.

The data on maternal body weight, maternal food consumption, maternal organ weights, numbers of corpora lutea, implants, live fetuses and dead embryos/fetuses (resorption, placental remnants, macerated fetuses), sex ratio, fetal body weight and the number of ossified sacro-caudal vertebrae in fetuses were analyzed generally on a litter basis [4] and statistically as follows. First, the data were tested by Bartlett's test for homogeneity of variances [5]. When the variances were homogeneous, Dunnett's test [6] was performed to compare the mean in the control group with that in each dosage group. When the variances were heterogeneous, a Dunnett type test [7, 8] which is based on ranked data was performed to compare the mean rank in the control group with that in each dosage group.

The data on frequencies of pre- and post-implantation loss, external and visceral abnormalities and variations of the fetuses, abnormal placentae and each external and visceral abnormality of the fetuses were transformed into ranked data as one experimental unit and were analyzed statistically as follows. The mean rank in the control group was compared with that in each dosage group utilizing a Dunnett type test [7, 8] which is based on ranked data.

The data on frequencies of skeletal abnormalities and variations of the fetuses were transformed into ranked data as one experimental unit and were analyzed statistically as follows. The Wilcoxon test was performed to compare the mean rank in the control group with that in each dosage group.

Bartlett's test was conducted at the significance level of 0.05, and the other tests were conducted at the two tailed significance levels of 0.05 and 0.01. Dunnett's test was performed using the SAS function PROBMC [9].

Statistical analysis was not performed for general signs or necropsy data in dams.

Results

Mortality: No mortality occurred in this study. Dose-related abortion was observed with two dams in the 300 mg/kg/day group and one dam in the 60 mg/kg/day group aborting their litters after the end of the TAK-375 administration period. One of the 300 mg/kg/day dams that aborted demonstrated marked weight loss during and after the dosing period while the other dams were not noted to have significant weight loss prior to abortion.

Clinical signs: Clinical observation indicated toxicity was not observed in rabbits treated with ≤ 60 mg/kg/day. Observations indicative of toxicity in dams treated with 300 mg/kg/day TAK-375 consisted of decreased spontaneous activity, creeping, lying on one side, rigidity, convulsions, myosis and a decrease in the amount of feces.

Body weight: Body weight was not affected in dams treated with ≤ 60 mg/kg/day TAK-375. Body weight gain was suppressed significantly in 300 mg/kg/day rabbits during the initial 5 days of the study but appeared to rebound after cessation of dosing in this group.

Food consumption: Food consumption was not affected in dams treated with ≤ 60 mg/kg/day TAK-375. Food consumption was similarly decreased significantly in the 300 mg/kg/day rabbits during the treatment period with a significant increase in food intake with cessation of dosing in this group.

Toxicokinetics: An additional study of TAK-375 and metabolites was completed in a separate study M-11-00188. For the present study, the Sponsor evaluated plasma concentrations of TAK-375 only and the Sponsor's summary table is reproduced below:

Dose (mg/kg/day)	No. of dosing (day of gestation)	T _{max} (h)	C _{max} (µg/mL)	AUC 0-24h (µg·h/mL)
12	1st (day 6)	0.3 (0.0)	1.12 (0.25)	5.2 (0.7)
	13th (day 18)	0.3 (0.0)	1.27 (0.37)	4.7 (0.8)
60	1st (day 6)	0.3 (0.0)	26.01 (1.74)	25.9 (1.7)
	13th (day 18)	0.3 (0.0)	23.96 (0.73)	22.0 (2.9)
300	1st (day 6)	0.5 (0.4)	73.56 (17.17)	237.3 (43.1)
	13th (day 18)	0.3 (0.1)	58.01 (28.96)	111.5 (31.9)

Mean (S.D.).

TAK-375 exposure (AUC₀₋₂₄) demonstrates dose-dependent increases which are remarkably linear after 13 days of dosing while C_{max} is slightly supra-proportional with increasing dose. Decreased exposure with repeated dosing is associated with the 300 mg/kg/day level but is not apparent with doses ≤ 60 mg/kg/day. T_{max} with oral dosing was consistently under 0.5 hr and did not change with repeated dosing.

Terminal and necropsic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): No abnormalities were observed with necropsy. Liver and adrenal gland weight was not affected by treatment. No differences between the groups were noted when examining the placenta, amnion or amniotic fluid. The numbers of corpora lutea and implantation sites were not different between treated groups and control animals indicating no alteration of pre-implantation loss. Post-implantation loss appeared to be somewhat increased with in dams treated with 300 mg/kg/day TAK-375 when compared with control (17.1 ± 25.3% vs. 6.4 ± 8.8%, respectively) though this did not achieve statistical significance. The number of live fetuses upon C-section was not different between treatment groups nor was sex ratio or body weight affected.

Offspring (malformations, variations, etc.): No statistically significant increases in malformations or variations were observed in litters of dams treated with 300 mg/kg/day TAK-375 when compared with control litters upon external, visceral or skeletal examination. Litters of intermediate doses were not examined as a result.

Observed Fetal Malformations and Variations

	Maternal TAK-375 dose (mg/kg/day)			
	0	12	60	300
External Findings				
# fetuses examined	141	146	163	117
Malformations (%)	0.4 ± 1.9	0	1.5 ± 4.9	0
Visceral Findings				
# fetuses examined	141	146	163	117
Malformations (%)	0.4 ± 1.9	0	0	0.8 ± 3.2
Diaphragmatic hernia	0	0	0	0.8 (1)

Adhesion of the hepatic lobe	0	0	0	0.8 (1)
Variations (%)	0	1.1 ± 4.6	0	0
Skeletal Findings				
# fetuses examined	141	NE	NE	117
Malformations (%)	1.8 ± 4.3	NE	NE	0
Variations (%)	25.9 ± 26.9	NE	NE	40.7 ± 30.3
Irregular lumbar rib	23.0 (12)			39.1 (13)
Shortening of 12 th rib	0			0.9 (1)
Dumb-bell-shaped cervical vt., body	0			0.9 (1)
Note: Only fetal abnormalities which appear increased with higher doses are specifically listed in table (), Numbers in parenthesis indicate the number of litters affected No findings were statistically significant ($p \leq 0.05$)				

The apparent increase in the frequency of observation of lumbar ribs in fetuses from 300 mg/kg/day litters when compared to control litters is not statistically significant and is within the range of historical control incidence in this species for this lab (21.0 – 49.6%) which was provided as an Appendix to the study.

In agreement with the Sponsor, the maternal NOAEL for the study is considered to be 60 mg/kg/day due to clinical signs and transient reduction in body weight observed at the 300 mg/kg/day level in dams while the embryofetal NOAEL is 300 mg/kg/day. The maternal plasma TAK-375 exposure associated with the embryofetal NOAEL is 58.0 µg/mL and 111.5 µg•h/mL (C_{max} and AUC_{0-24h} , respectively).

Study title: Plasma Concentrations of TAK-375 and its Metabolites in Pregnant Rabbits

Key study findings:

- Pregnant 17-week old C57BL/6J rats analogous to those used in the Segment II reproductive toxicology study described above were administered TAK-375 at doses of 300 mg/kg/day for gestation days 6-18 to determine the toxicokinetic parameters of TAK-375 and its metabolites M-II (2S, 8S [active]), M-I, M-III, and M-IV.
- At the NOAEL dose determined in the previous embryofetal development study, 300 mg/kg/day, maternal TAK-375 exposures were 35,514 ng/mL and 71,463 ng•h/mL (C_{max} and AUC_{0-24} , respectively) at the end of the dosing period while the associated M-II (2S, 8S) exposure was 3,368 ng/mL and 23,847 ng•h/mL (C_{max} and AUC_{0-24} respectively). See RESULTS for the results of (inactive) metabolites M-I, M-III and M-IV.
- Comparison of exposure at the start of TAK-375 treatment with the end of treatment revealed a 49% reduction in exposure to TAK-375 (1st dose $AUC_{0-24} = 138,889$ ng•h/mL, 13th dose $AUC_{0-24} = 71,463$ ng•h/mL) and a 73% reduction in exposure to M-II (88,714 ng•h/mL vs. 23,847 ng•h/mL) with a 163% increase in M-I exposure (52,819 ng•h/mL vs. 139,290 ng•h/mL). Other metabolites appeared generally unchanged with repeated dosing.

- When NOAEL exposure values are compared to human values obtained in a clinical study with the proposed 8 mg tablet, the margin of exposure at the NOAEL dose at the end of the treatment period provides for a 24,159-fold and 27,069-fold (C_{max} and AUC, respectively) margin of safety for TAK-375 while for M-II (2S, 8S) this margin is 62-fold and 104-fold.

Study no.: M-11-00188

Volume #, and page #: N/A

Conducting laboratory and location: Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd., Yamaguchi, Japan

Date of study initiation: 8/19/1999

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: TAK-375 Lot # M375-013, purity ≥ 1 . Content of the lot and the stability and homogeneity of the dosing solution was verified and documented in the Appendix.

Methods

Doses: A single TAK-375 dose of 300 mg/kg/day was administered during gestation days 6 – 18 to pregnant dams.

Species/strain: $\square$ JW $\square$ rabbits

Age: 17-weeks; Wt: 3.01 – 4.08 kg at time of dosing initiation

Number/sex/group: 5 mated females

Route, formulation, volume, and infusion rate: Oral gavage with test article dissolved in 0.5 w/v% MC and given as 10 mL/kg

Satellite groups used for toxicokinetics: Not done, TK group are main animals.

Study design: From Sponsor's submission (directly)

Group	Test article	Dosage level (mg/kg/day)	Dosage volume (mL/kg)	Concentration of dosing suspension (w/v%)	Number of animals	
					Copulated	Pregnant
1	TAK-375	300	10	3	5	5

Parameters and endpoints evaluated:

General signs: Mortality and clinical signs assessed once daily during non-treatment period and 3 times daily during treatment period (before, 30-45 min after dosing and 1-2 hr after dosing).

Body weight: Assessed on gestation days 0, 6, 8, 10, 12, 14, 16, 18, and 19.

Food consumption: 24-hr value determined on gestation days 0, 6, 8, 10, 12, 14, 16 and 18.

Necropsy: Dams were killed with a euthanasia solution of Nembutal on Day 20 of gestation. Impregnation was confirmed and all main internal organs were examined macroscopically for abnormalities.

Toxicokinetics: Blood was obtained from the auricular vein of 5 animals on Day 6, 11 and 18 (1st, 6th, and 13th dose) at 0.25, 0.5, 1, 3, 6 and 24 hr after dosing. Plasma samples were analyzed for concentrations of TAK-375, M-I, M-II, M-III and M-IV by $\square$ at each time-point in 3

animals. The M-II epimer ratio [(2*S*, 8*S*) vs. (2*R*, 8*S*)] at 1 and 3 hr after dosing was also determined.

Results

General: No mortality was observed and none of the 5 mated rabbits aborted. Clinical signs were observed in all dams and were characterized by creeping and a decrease in spontaneous activity. A reduction in feces was noted shortly after the beginning of the dosing period and continued for the duration of treatment. Body weight gain was suppressed and paralleled the reduction in food consumption that was observed throughout the dosing period. Necropsy did not reveal any abnormalities in examined dams.

Toxicokinetics:

The Sponsor's table listing the toxicokinetic results is reproduced below.

Toxicokinetic measurements of TAK-375, M-I, M-II, M-III, and M-IV in the maternal plasma of pregnant rabbits administered 300 mg/kg/day TAK-375 by oral gavage

Dose (mg/kg/day)	Analyte	No. of dosing	Female (n=3)		
			Tmax (h)	Cmax (ng/mL)	AUC 0-24h (ng·h/mL)
300	TAK-375	1st	0.3 (0.1)	63902 (9801)	138859 (28819)
		6th	0.8 (0.3)	35065 (4722)	89444 (7838)
		13th	0.3 (0)	35514 (3604)	71463 (18003)
	M-I	1st	0.5 (0)	19259 (1656)	52819 (6097)
		6th	0.5 (0)	26163 (9775)	72529 (24865)
		13th	0.3 (0.1)	49197 (5598)	139290 (63373)
	M-II	1st	2.3 (1.2)	13230 (2813)	88714 (44863)
		6th	2.3 (1.2)	10280 (5013)	56012 (24383)
		13th	2.3 (1.2)	3368 (1491)	23847 (9548)
	M-III	1st	1.0 (0)	6810 (367)	21018 (2095)
		6th	1.0 (0)	3457 (1502)	16029 (3656)
		13th	1.5 (1.3)	5233 (1603)	21203 (9878)
	M-IV	1st	3.0 (0)	5326 (976)	37498 (14738)
		6th	3.0 (0)	4169 (1989)	22928 (6129)
		13th	3.0 (0)	2668 (1465)	16873 (5985)

Clear reduction in exposure to TAK-375 (↓49%) and M-II (↓73%) was apparent with repeated TAK-375 administration while an increase in M-I was observed (↑163%) over this same period. Significant exposure was observed to all human TAK-375 metabolites. Margins of safety for TAK-375 and the active M-II metabolite determined in the maternal plasma at the embryo-fetal NOAEL are described in the following table:

Toxicokinetic Parameters (Day 18/13th Dose) and Margin of Safety of Human Exposure[†]			
Dose	Anylate	C _{max} (ng/mL)	AUC
300 mg/kg/day	TAK-375	35,514 (6,123-fold)	71,463 (7,602-fold)
	M-II	3,368 (54-fold)	23,847 (99-fold)

[†], Derived from Clinical Study in adults and elderly (01-01-TL-375-003) in which 16 mg Ramelteon was administered to subjects. Exposure for the 8 mg intended dose of Ramelteon is expected to be linear over the dose-range and therefore the Sponsor's estimate of C_{max} and AUC₀₋₂₄ at 8 mg is: TAK-375 (5.8 ng/mL, 9.4 ng·h/mL), M-II (62.5 ng/mL, 241.3 ng·h/mL)

Prenatal and postnatal development

Study title: Range-Finding Study for Effect of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function in Rats

Key study findings:

- Dams were administered vehicle (0.5 w/v% methylcellulose) or TAK-375 at 30, 100 or 300 mg/kg/day by oral gavage once daily from Days 6 of pregnancy to Postnatal Day 6. Surviving offspring were culled to 4 pups/sex/group and examined for external abnormalities, body weight, clinical signs and viability and abnormalities from necropsy exam.
- **Dams:** Toxicity associated with TAK-375 dosing was limited to the 300 mg/kg/day group which demonstrated decreased spontaneous locomotion (Days 6-11), salivation (Days 11-22), decreased body weight (Days 10-14) and body weight gain (Day 8) when compared with dams administered the vehicle control. No alterations in adrenal weight, gestational parameters or implantation site numbers were noted in treated groups.
- **F₁ offspring:** No alterations in birth number, sex ratio, viability, clinical signs, external abnormalities or alterations on necropsy exam were associated with TAK-375 treatment of dams up to the highest dose tested, 300 mg/kg/day.
- **NOAEL(maternal toxicity) = 100 mg/kg/day, while NOAEL(F₁ offspring) = 300 mg/kg/day.**

Study no.: M-11-00294

Volume #, and page #: N/A

Conducting laboratory and location: [

1

Date of study initiation: May 6, 1999

GLP compliance: Yes (Japan)

QA reports: yes (X) no ()

Drug, lot #, and % purity: TAK-375, Lot #M375-013, [] % purity with COA in appendix; stability and homogeneity of test article in 0.5 w/v% methylcellulose suspension confirmed in appendix.

Methods

Doses: Vehicle (0.5 w/v% methylcellulose), TAK-375 30, 100 or 300 mg/kg/day

Species/strain: Sprague-Dawley rat, — CD(SD) IGS, SPF)

Number/sex/group: 7 dams/dose group; F₁ offspring culled to 4 pups/sex/litter/dose group

Route, formulation, volume, and infusion rate: Oral gavage of test article suspended in 0.5 w/v% methylcellulose

Satellite groups used for toxicokinetics: Not done

Study design: Female rats were mated with males and the appearance of a vaginal plug or detection of sperm on analysis of vaginal smear was considered Day 0 of pregnancy.

Females were housed separately after mating and administered vehicle or TAK-375 at various doses by oral gavage from Day 6 of pregnancy to postnatal Day 6 (23-25 days of administration). Dams were sacrificed 7 days after delivery of offspring and were necropsied. Offspring were culled to 4 animals/sex for each litter. Offspring remaining after culling were sacrificed on (PND7) and subject to postmortem evaluation. Dose groups are described in the following table.

Test group	Dosage level (mg/kg/day)	Concentration (w/v%)	No. of females which copulated (Animal No.)
1. Vehicle control ^{a)}	0	0	7 (F01051 - F01057) ^{b)}
2. TAK-375	30	0.3	7 (F02151 - F02157)
3. TAK-375	100	1	7 (F03251 - F03257)
4. TAK-375	300	3	7 (F04351 - F04357)

a) 0.5 w/v% methylcellulose solution.

b) Animal No. F01052 failed to conceive.

Parameters and endpoints evaluated:

Dams:

Mortality and general signs once daily during non-dosing period and once before and once after daily administration of drug. Dams were weighed on Days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of pregnancy, on the day of delivery (parturition) and Day 4 and Day 7 after delivery. Food consumption was monitored on Days 0, 6, 8, 10, 12, 14, 16, 18 and 19 of pregnancy and on Parturition Day (Day 0), Day 4 and Day 6 after delivery. Dams that had not delivered by Day 25 of pregnancy were sacrificed and necropsied. Nursing conditions observed daily until PND7. Dams were sacrificed by exsanguination under ether anesthesia and necropsied. Implantation sites were counted and the adrenal gland was removed and weighed. Fertility parameters calculated included the Gestational index (%) (#dams with live offspring/#dams pregnant)x100 and Gestational length (days).

Offspring (F₁):

The number of live and stillbirth offspring were counted upon parturition. Stillbirths were fixed and preserved in NBF. Live offspring were assessed for gender and external abnormalities. Mortality and clinical signs were assessed over Days 0 – PND6. Weight was taken on PND0, PND4 and PND7. Offspring were sacrificed on PND7 by exsanguination under ether anesthesia and necropsied. Parameters calculated included Birth index (%): #offspring on parturition day/#implantation scars)x100, external abnormalities incidence, sex ratio, Viability index PND4 (%), Viability index PND7 (%).

Results

F₀ in-life: There were no dams found dead, sacrificed due to poor condition, dams with aborted litters or premature delivery in any treatment or vehicle control group. Abnormal signs were not found in the vehicle group or TAK-375 30 or 100 mg/kg/day groups but at 300 mg/kg/day TAK-375 a decrease in spontaneous locomotor activity was observed in 6/7 dams sporadically over Days 6 – 11 of pregnancy. Salivation was noted sporadically in all pregnant females in this group at various times over Days 11 – 22 of pregnancy and after parturition as well. The timing of the salivation in relation to dosing was not described. Body weight was unaffected in vehicle groups or TAK-375 groups at 30 or 100 mg/kg/day but at 300 mg/kg/day body weight was significantly below control on Days 10 – 14 of pregnancy. Body weight gain

was reduced in this group only Day 8 of pregnancy. Food consumption was unaffected in the TAK-375 30 mg/kg/day group but was significantly increased in the 100 mg/kg/day group on Day 19 of pregnancy. In contrast, the 300 mg/kg/day TAK-375 group showed a significant decrease in food consumption on Days 6 – 12 while it was higher than controls on Day 19. Therefore the significant increase in food consumption occurring in ≥ 100 mg/kg/day TAK-375 dose levels was only on Day 19 while the body weight decreases in the 300 mg/kg/day group were more durable and probably treatment-related. Gestation length was not different from vehicle controls and Gestational index was 100% in all groups. The sponsor noted that the dam in the 30 mg/kg/day group in which all offspring died exhibited “poor nest-making ability”. No lactation abnormalities were noted in this animal or any other from the TAK-375 treatment groups or vehicle controls.

F₀ necropsy: No abnormalities were observed in any dam from vehicle or TAK-375 groups. Adrenal weights were not different between treatment groups and vehicle controls. Implantation site numbers were not significantly different between any treated groups and vehicle controls.

F₁ physical development: External abnormalities were not elevated in litters of TAK-375 treated dams as compared to vehicle control litters. Body weight was unchanged. No abnormalities were observed upon necropsy exam.

F₁ behavioral evaluation: No treatment-related differences in clinical signs were observed between TAK-375 litters and vehicle control litters. One litter of a 30 mg/kg/day TAK-375 treated dam showed evidence of hypothermia and reduced locomotor activity, all of which died by PND3. As this only occurred in a single litter of a low-dose group this was not considered to be test-article related.

F₁ reproduction: Not conducted.

F₂ findings: N/A

Study title: Effects of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function, in Rats

Key study findings:

Pregnant Sprague-Dawley rats (F₀) were administered vehicle (0.5 w/v% methylcellulose), or TAK-375 at 30, 100 or 300 mg/kg by oral gavage from Day 6 of pregnancy to Day 21 of lactation. The study design appeared to be appropriate according to the current state of the science. Key findings include:

F₀ Dams

- No effects on F₀ with TAK-375 dose of 30 mg/kg. At 100 mg/kg TAK-375, **increased adrenal gland weight** (absolute and relative) was observed as well as **sporadic findings of reduced body weight and body weight gain** during the lactation period which was also reflected in **decreased food consumption**. At 300 mg/kg TAK-375 was associated with a **decrease in locomotor activity**,

increase in **adrenal gland weight** (absolute and relative), **reduction in body weight** during pregnancy as well as during the lactation period with a concomitant **reduction in food consumption**. In a minority of dams in the 300 mg/kg group evidence of **poor nest-building** was observed (3/20 dams) as well as **impaired lactation** (2/20 dams) which resulted in the loss of all offspring in these 3 litters. A single dam in the 30 mg/kg group demonstrated poor nest-building and offspring from this litter died as well shortly after birth.

- No statistically significant effects on reproduction parameters were observed when comparing vehicle-treated dams with those receiving TAK-375 doses up to 300 mg/kg.

F₁ offspring

- Effects on F₁ offspring were not observed in the 30 mg/kg group. The 100 mg/kg group offspring demonstrated **reduced body weight** both during weaning and after weaning with males having a longer suppression of body weight than females post-weaning. The 300 mg/kg group offspring demonstrated a **greater reduction in body weight** which was suppressed longer in females than in the lower dose group. Evidence of delayed maturation included a reduced rate of differentiation in the 300 mg/kg group for **eruption of lower incisors** than in controls and a **reduced acquisition of the righting reflex** over the first two days of reflex observation when compared to control group offspring. A test of emotional responses indicated **altered emotional response** in the 300 mg/kg offspring defined as an increased latency to movement though other parameters measured in this assessment were no different between offspring of different groups and controls.
- Postmortem examination of F₁ offspring at weaning revealed an **increased incidence of diaphragmatic hernia** in offspring of the 300 mg/kg group.
- F₁ offspring selected for evaluation of reproductive function did not reveal any significant differences between offspring of the control group and those of TAK-375-treated groups.

F₂ offspring

- No differences between F₂ offspring of TAK-375-treated groups and offspring of the control group were observed on body weight, external exam or upon necropsy.
- Overall, reproductive ability was not impaired in either the F₀ or F₁ generation though the F₀ dams demonstrated impaired nest-building and lactation with the 300 mg/kg dose. Therefore, the NOAEL (F₀, F₁ reproductive toxicity) = 100 mg/kg (F₁) and 300 mg/kg (F₂), respectively. In the F₀ generation, the NOAEL (F₀ maternal toxicity) = 100 mg/kg due to effects on body weight, food consumption, and adrenal gland weight. F₁ offspring postnatal developmental effects including reduced body weight were observed in offspring of the 100 and 300 mg/kg groups as well as observations of delays in morphologic differentiation rate, reflexes, and emotional alterations were observed with F₁ offspring of the 300 mg/kg group. F₁ offspring of the 300 mg/kg group also had a higher incidence of a visceral abnormality: diaphragmatic hernia (a finding also observed with doses ≥ 150 mg/kg in a Segment II embryofetal toxicity study). Therefore the NOAEL (F₁ developmental toxicity) = 30 mg/kg. The F₂

generation did not appear different between treatment groups in a limited assessment including body weight, external examination and necropsy exam.

Study no.: M-11-00271.001

Volume #, and page #: N/A

Conducting laboratory and location: τ

$\downarrow$

Date of study initiation: Feb. 10, 2000

GLP compliance: Yes (Japan)

QA reports: yes (X) no ()

Drug, lot #, and % purity: TAK-375, Lot #M375-013, purity τ $\downarrow$ %, COA in Appendices. Stability and homogeneity are acceptable according to data supplied in Appendices.

Methods

Doses: Vehicle (0.5 w/v% methylcellulose) or TAK-375 30, 100 or 300 mg/kg/day.

Species/strain: Sprague-Dawley rat τ $\downarrow$ CD(SD)IGS, SF

Number/sex/group: 19-20 pregnant dams/dose group; F₁ offspring culled to 4 pups/sex/litter/dose group

Route, formulation, volume, and infusion rate: Oral gavage of test article suspended in 0.5 w/v% methylcellulose

Satellite groups used for toxicokinetics: Not done.

Study design: Females were mated with males and the day on which a pregnancy plug was observed or vaginal smear positive for sperm was considered Day 0 for pregnancy. Pregnant females were administered TAK-375 or vehicle daily for 37-39 days from Day 6 of pregnancy to PND21 as shown in the Sponsor's table below.

Test group	Dosage level (mg/kg/day)	Concentration (w/v%)	No. of females which copulated (Animal No.)
1. Vehicle control ^{a)}	0	0	20 (F01151 - F01170)
2. TAK-375	30	0.3	20 (F02251 - F02270) ^{b)}
3. TAK-375	100	1	20 (F03351 - F03370)
4. TAK-375	300	3	20 (F04451 - F04470)

a) 0.5 w/v% methylcellulose solution.

b) Animal No. F02268 failed to conceive.

Offspring of dams were culled by random selection at PND4 to 4/sex/litter/group prior to further observations. When litter size was 8 offspring or less all the pups were retained in the study. After selection, pups were examined for developmental differences in morphologic differentiation until PND21. All stillbirths and offspring dying prior to weaning on Day 21 were fixed and retained for analysis. Two (2) offspring/sex/litter were selected for reflex assessments during the first 6 weeks while the remaining 2 offspring/sex/litter were sacrificed on PND21 and necropsied. One offspring/sex/litter was selected for evaluation of learning ability during Weeks 6 to 7 and following confirmation of reproductive function was sacrificed in Week 21. One F₁ offspring/sex/litter was selected for assessment of emotional response followed by evaluation of reproductive function. Emotional responses were examined during Postnatal Week 4 using the open field method while reproductive function was assessed in females by assessment of the estrous cycle from Week 11 through Week 16 or at the time of copulation during Weeks 13-16. Males were sacrificed and necropsied after copulation while

females were allowed to deliver offspring and wean prior to sacrifice and necropsy. Offspring (F2) were weaned at 21 days and sacrificed and necropsied.

Parameters and endpoints evaluated: *Dams:* Dams were observed for mortality and clinical signs once prior to dosing and once after dosing on dosing days while this evaluation was conducted only once daily during the non-dosing period. Body weight was recorded on Days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of pregnancy, at parturition and on Days 4, 7, 11, 14, 17 and 21 after delivery. Food consumption was calculated on Days 0, 6, 8, 10, 12, 14, 16, 18 and 19 of pregnancy and Days 0, 4, 7, 11 and 14 days after delivery of pups. Dams were evaluated for parturition 3 times on Day 21 of pregnancy and continued until delivery. Lack of delivery by Day 25 necessitated sacrifice and examination of the uterus; the ovaries and uterus were fixed in formalin if no implantations were noted. Lactation conditions were observed daily for 21 days after delivery. Dams were sacrificed at 21 days after delivery by exsanguination under anesthesia. Implantation sites were counted, adrenal glands were weighed and organs in which abnormalities were found on gross examination were preserved. *F1 Offspring:* Live offspring and stillbirths were counted after delivery. Live offspring were sexed and examined for external abnormalities. Stillbirths were fixed and examined where possible. Offspring were observed for mortality and clinical observations once daily with dead offspring fixed and examined. Culling of offspring was conducted on PND4 in litters of > 8 offspring such that 4 offspring/sex/litter remained for further analysis. Offspring were weighed on PND0, 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70 and PND77. Morphologic differentiation was evaluated over a period up to PND41 (lower incisor eruption at PND10, separation of eyelids at PND14, opening of vaginal orifice at PND33, cleavage of the balanopreputial gland at PND41. 2 offspring/sex/litter were sacrificed and necropsied after weaning on PND21 while the remaining 2 offspring/sex/litter were further for reflexes (righting reflex at PND6, negative geotaxis at PND10, free-fall reflex, corneal reflex and pinna reflex at PND17. If reflexes were not observed on those days the animal was followed until up to sacrifice for development of the reflex. One offspring/sex/litter was then examined for learning ability using multiple water T-maze test while the remaining one offspring/sex/litter was examined for emotional development during Week 4. Emotional responses were examined during Postnatal Week 4 using the open field method. Reproductive function was assessed in F1 offspring used for both learning ability and emotional development with the estrous cycle in females being examined starting in Week 11 and continuing through copulation. Mating was allowed during Week 13-16 with sacrifice of F1 males after copulation. The testes and epididymides were fixed and examined microscopically. Females were examined for mortality and clinical signs and weighed on Days 0, 6, 14, and 20 of pregnancy and Days 0 (parturition), 4, 7, 14 and 21 after delivery. Dams were monitored for parturition 3 times daily starting on Day 21 of pregnancy. Lactation conditions were monitored for 21 days after delivery. F1 dams were sacrificed on Day 21 by exsanguination under anesthesia and necropsied with implantation sites counted. Organs with macroscopic aberrations were retained and 3 of the same tissues from control animals were taken for comparison. *F2 Offspring:* Live offspring and stillbirths were counted. Live offspring were sexed and examined for external abnormalities while stillbirths were preserved in fixative. Offspring were examined for mortality and clinical signs once daily for 21 days (until weaning). Random selection of 4 offspring/sex/litter was conducted on PND4 and the other offspring were sacrificed. Offspring remaining were weighted on PND0, and PNDs 4, 7, 14, and 21. F2 offspring were sacrificed on PND21 and necropsied. Organs

which had macroscopic findings were compared to comparable tissues taken from 3 control animals.

Results

F₀ in-life: No dams died with dosing, aborted or delivered prematurely. Clinical signs were observed with dosing of ≥ 100 mg/kg/day and included salivation (at 100 mg/kg/day) throughout pregnancy and during the post-delivery period while **reduced locomotor activity** was observed in dams given 300 mg/kg/day sporadically throughout the dosing period until parturition.

Body weight was unchanged in dams given 30 mg/kg/day but **body weight was decreased** significantly in 100 mg/kg/day dams on Days 10 and 14 after delivery while in dams given 300 mg/kg/day reduced body weight was observed during pregnancy (Days 10-20) and after delivery (Days 7-21 after delivery).

Body Weight			
Dose (mg/kg)			
0	30	100	300
---	---	Pregnancy: No change Lactation: D11 & D14 (↓4%)	Pregnancy D10 - 20 (↓5-7%); Lactation D7-21 (↓5-8%)

Body weight gain was not different from control with administration of 30 mg/kg/day but at 100 mg/kg/day was associated with transiently reduced body weight gain as measured on Day 8 of pregnancy and on Day 11 after delivery while at 300 mg/kg/day was associated with body weight gain reductions on Day 8 and 10 of pregnancy and Days 4 and 11 after delivery. Significantly increased body weight gain was observed on Day 17 after delivery but this was not considered adverse.

Food consumption was unchanged with the 30 mg/kg/day dose but was associated with **decreased food consumption** sporadically after delivery in the 100 mg/kg/day group and during pregnancy (Days 6-14 and 18 of pregnancy) and Days 7-14 after delivery.

Food Consumption			
Dose (mg/kg)			
0	30	100	300
---	---	Pregnancy: No change Lactation: D11 & D14 (↓6-8%)	Pregnancy D6 - 14 & D18 (↓10-29%); Lactation D7, 11 & 14 (↓12-13%)

Gestation length was not altered in a dose-dependent manner though an increase was noted in the 30 mg/kg/day group. No differences were observed in the number of live offspring at birth, the sex ratio, birth index, viability index or weaning indexes in the 30 or 100 mg/kg/day groups. Viability appeared to be lower in the 300 mg/kg/day group overall but no differences were observed in the other parameters in this group. No treatment-related external abnormalities were apparent. Lactation and nest building was unaffected in the 100 mg/kg/day group but **nest building was considered poor** in 1/20 dams in the 30 mg/kg/day group and 2/20 dams in the 300 mg/kg/day group while these animals also had **incomplete nipple**

development as did another female in the 300 mg/kg/day group (3/20 dams total). All the offspring in the litters of these 3 females died within 4 days of delivery.

Reproductive Function (F₀)				
	Dose (mg/kg)			
	0	30	100	300
# Dams	20	20	19	20
Gestation length	n.d.			
Implantation site #	n.d.			
Gestation index	n.d.			
# Live offspring	n.d.			
Sex ratio	n.d.			
Stillbirths	n.d.			
Birth index	n.d.			
Viability index	98.4 ± 3.5%	91.7 ± 22.6% ^a	97.6 ± 4.7%	83.1 ± 36.3% ^b
Weaning index	n.d.			
%External abnorm.	n.d.			
Observations				
Faulty Nest Building	---	1/20	---	3/20
Nipple Function Impaired	---	---	---	2/20

^a, 1 dam had all offspring die; ^b, 3 dams had all offspring die (all in dams with described observations of faulty nest-building and impaired nipple function).

F₀ necropsy: No treatment-related macroscopic abnormalities were observed upon necropsy including the 1 dam given 30 mg/kg/day and the 3 dams given 300 mg/kg/day for which all offspring died. Adrenal weight was unchanged in the 30 mg/kg/day dams but was significantly increased (relative and absolute weights) with TAK-375 dosing ≥ 100 mg/kg/day.

Organ Weight (% change relative to vehicle group)				
	Dose (mg/kg)			
	0	30	100	300
Adrenal gland				
Absolute	---	n.d.	↑9%*	↑28%**
Relative	---	n.d.	↑13%*	↑31%**

*p<0.05, **p<0.01

F₁ physical development: No treatment-related clinical observations were noted with dosing ≤ 100 mg/kg/day. All offspring of 3/20 dams treated with 300 mg/kg/day TAK-375 died within 4 days with 1 offspring/litter displaying evidence of decreased locomotor activity and hypothermia prior to death. In dams with surviving litters, 1 offspring demonstrated an enlarged head and unopened eyes up to 21 days after birth. The significance of this finding is unclear as it occurred in only one animal in one litter at the high dose.

Body weight was decreased in both male and female offspring of dams treated with ≥ 100 mg/kg/day TAK-375 up to weaning. This decreased weight continued in females to 42 days after birth while in males this was noted out to 77 days after birth.

F1 Offspring Body Weight				
	Dose (mg/kg)			
	0	30	100	300

	M	F	M	F	M	F	M	F
Pre-weaning	---	---	n.d.	n.d.	PND14,21: ↓7%*	PND14,21: ↓6-7%*	PND7,14,21: ↓8*-13%**	PND14,21: ↓13%**
Post-weaning	---	---	n.d.	n.d.	PND28-77: ↓6-7%**	PND28: ↓5%*	PND28-77: ↓10-12%**	PND28-42: ↓6-11%**
Duration of weight suppression	---	---	n.d.	n.d.	PND 77	PND 28	PND 77	PND 42
n.d. Not significantly different from control; *p<0.05 vs. control, **p<0.01 vs. control								

Differentiation rate for auricle separation, separation of the eyelids, opening of the vaginal orifice and balanopreputial gland was similar between offspring of treated and untreated dams. The **differentiation rate for the eruption of the lower incisors, however, was lower in offspring of the 300 mg/kg/day group** while this was not observed with offspring of dams administered lower doses of TAK-375.

Morphologic Differentiation					
	Postnatal Day (PND)	Dose Group (mg/kg)			
		0	30	100	300
Eruption of Lower Incisors (%)	10	32	27	20	15*
	11	73	71	67	51*
	12	96	94	93	91
	13	100	98	100	97
	14	100	99	100	100
	15	100	100	100	100

F₁ behavioral evaluation: *Reflexes:* Assessment of negative geotaxis, free-fall reflex, corneal reflex and pinna reflex did not demonstrate a dose-dependent alteration of reflexes though **righting reflex was dose dependently prolonged at 6 and 7 days after birth in the offspring of the 300 mg/kg/day group.** *Learning ability:* No differences in measured parameters in the water T-maze were observed in offspring of the TAK-375-treated dams when compared to those offspring from vehicle-treated dams. *Emotional response:* Prolonged latency to ambulation was observed in the open field test in both males and females in the 300 mg/kg/day offspring when compared to control offspring. No differences were observed in ambulation, rearing, preening, grooming, defecation or urination.

Reflex Evaluation					
	Postnatal Day (PND)	Dose Group (mg/kg)			
		0	30	100	300
Righting Reflex (%)	6	75	78	68	41**
	7	98	94	91	83**
	8	99	100	98	94
	9	100	100	100	99
	10	100	100	100	100
Learning Ability Evaluation					
	Postnatal Day (PND)	Dose Group (mg/kg)			
		0	30	100	300

Multiple Water T-Maze	6-7 Weeks	No differences			
Emotional Response Evaluation					
	Postnatal Day (PND)	Dose Group (mg/kg)			
Open Field	4 Weeks	0	30	100	300
Latency (sec)		M / F	M / F	M / F	M / F
		14±14 / 10±11	15±10 / 9±7	19±39 / 19±20	30±42* / 33±53*
*p<0.05; **p<0.01					

F₁ reproduction: No differences were observed between offspring of TAK-375-treated dams versus control treated dams in gestation duration, number of implantation sites or gestation index (which was 100% for all groups).

F₁ postmortem examinations: *Stillbirth or died before weaning:* No gross necropsy findings. Skeletal abnormalities observed in 1 offspring of 30 mg/kg group (supernumerary lumbar vertebra) and a short rib was noted in 1 offspring of the 300 mg/kg group. No significant differences were observed in skeletal abnormalities or variations, however, when compared with control group offspring. *Weaning (21 days after birth):* On gross necropsy examination, no abnormalities in vehicle or 100 mg/kg offspring. Microphthalmia observed in 1 offspring of the 30 mg/kg group. **Treatment-related effects appeared in the 300 mg/kg group offspring and involved diaphragmatic hernia** (2 males and 1 female). This had previously been observed in the fetuses of rats treated with 150 mg/kg TAK-375 in a Segment II embryofetal development study. No significant differences in skeletal abnormalities were observed in offspring of treated dams though single cases of fused rib, sacralization of lumbar vertebra and a short rib and lumbarization of sacral vertebra were seen in single animals in the 30, 100 and 300 mg/kg groups respectively. No significant differences in skeletal variations were noted. *End of study (21 Weeks after birth):* No apparent treatment effects. Sporadic findings of dilated renal pelvis noted in control and treated groups, one offspring per group maximum. Soft testes noted in 1 F1 offspring from the 30 mg/kg group. Soft and small testis and small epididymis observed in one offspring in the 300 mg/kg group.

Post Mortem Examination of F ₁ Offspring ^a								
	Dose							
	0		30		100		300	
	M	F	M	F	M	F	M	F
<i>Stillbirth/Dead Before Weaning</i>								
Necropsy exam								
No findings								
Skeletal exam								
# dams	9		7		6		6	
# examined	15		7		10		27	
Abnormality: Short rib	0		0		0		1	
<i>PND21 (weaning)</i>								

Necropsy exam								
# examined	41	39	39	33	41	39	32	32
Diaphragmatic hernia	0	0	0	0	0	0	2 (6.3%)	1 (3.1%)
Skeletal exam								
# examined	80		72		80		64	
Abnormalities								
Lumbarization of sacral vertebra	0		0		0		1	
Sacralization of lumbar vertebra	0		0		1		0	
Variations								
External sternal ossification site	7		6		7		11	
Short supernumerary rib	6		3		4		9	
Post-reproductive assessment or PNW 21 (PND147) ^b								
Necropsy exam								
# examined	40	38	36	35	40	40	34	33
Testis								
Small	0	-	1	-	1	-	1	-
Soft	0	-	2	-	1	-	1	-
Epididymis								
Small	0	-	1	-	1	-	0	-
Brain								
Anophthalmia (right & left)	0	0	0	0	0	0	0	1
Dilated cerebral ventricle	0	0	0	0	0	0	0	1
^a , Only observations which appear to be treatment-related or dose-related are shown								
^b , 1 F1 offspring/sex/litter was used for reproductive assessment (F2 generation) and males sacrificed after copulation while females sacrificed 21 days after delivery. Another F1 offspring/sex/litter was used for learning assessment, allowed to reproduce to assure function and sacrificed 21 weeks after birth.								

F₂ findings: F₂ offspring groups were not different based on viability parameters such as number of live offspring at birth, sex ratio, number of stillbirths, birth index, or weaning index. No abnormal were found on external examination which appeared to be related to drug treatment. No differences between vehicle control group and TAK-375 treated groups were observed in body weight from birth to weaning or in the necropsy exam. General signs of F₂ offspring which may be treatment-related included hypothermia which was observed in 1 F₂ offspring in 1 dam of the 100 mg/kg group and 2-4 offspring of a single F₁ dam in the 300 mg/kg group. The Sponsor did not consider this to be adverse as it was only observed in the F₂ offspring of single dams in the 100 and 300 mg/kg groups and this reviewer is in agreement.

2.6.6.7 Local tolerance

Three studies were submitted by the sponsor including an *in vitro* hemocompatibility study with human blood, an intravenous local tolerance study in the rabbit and a paravenous local tolerance study in the rabbit. As TAK-375 is intended to be administered by the oral route in the human and local tolerance is appropriately assessed in standard nonclinical toxicology studies with clinical observation and gross and microscopic exam of the gastrointestinal system, these studies are unnecessary for the NDA and are not reviewed.

2.6.6.8 Special toxicology studies

The effects of TAK-375 on observed rodent plasma hormone concentrations were assessed in a series of studies which are described below:

Study Title: Effects of TAK-375 on Melatonin and Thyroid Hormone Levels and On Histology of the Harderian Glands in Aged Mice

Key findings:

- TAK-375 was administered to aged mice (62-63 weeks) for 4 consecutive weeks to assess a potential mechanistic explanation for the increase in Harderian gland adenomas found in TAK-375-treated mice in a 2-year carcinogenicity bioassay.
- Administration of TAK-375 in doses ≥ 30 mg/kg/day produced a **marked increase in circulating plasma melatonin** levels and an increase in Harderian gland melatonin levels.
- TAK-375 administered up to 300 mg/kg/day did not affect circulating plasma thyroid hormone levels or affect the Harderian gland structurally. No histopathologic alterations were found nor was evidence, by PCNA immunohistochemistry, detected to suggest an effect of TAK-375 on proliferation of Harderian gland cells.

Study #: M-11-00709

Vol/Page: N/A

Conducting laboratory and location: Drug Safety Research Center, Takeda Chemical Industries, Ltd., Yamaguchi 743-8502 Japan

Date of study report: May 13, 2004

GLP compliance: No

QA report: Yes () No (X)

Test article: TAK-375 (lot # M375-028), purity stated as $\geq 98\%$, stability confirmed with studies on another TAK-375 lot.

Methods: Male and female C57BL/6J mice aged 62-63 weeks at the initiation of the study were used to probe the effects of TAK-375 on plasma and Harderian gland hormone levels in a repeat-dose study. Animals were administered TAK-375 at doses of 0 (0.5 w/v% methylcellulose), 3, 30 or 300 mg/kg/day for 4 weeks. After the last treatment, plasma was collected either 1 hr or 12-14 hr after the last TAK-375 administration at the time of necropsy. Plasma was analyzed for hormones including melatonin, TSH, T3 and T4 while the Harderian gland (pooled from 3 animals per sample) was assessed for levels of melatonin and gross and microscopic alterations observed. Plasma and Harderian gland tissue from two females in the control group and 2 males in the 3 mg/kg/day group were not analyzed due to the presence of

white foci or nodules on the Harderian gland. For reasons which are unclear, a number of animals were analyzed in these and other treatment conditions despite the same gross findings. Plasma and tissue hormone levels were assayed for hormones using commercially available kits for melatonin, TSH, T3, and T4. The anti-melatonin antibody used to quantify melatonin concentrations demonstrated minimal cross-reactivity for TAK-375 and metabolites, therefore this potential confound was not observed. Organ weights were assessed along with body weight for calculation of relative organ weights. Harderian glands obtained at necropsy were sectioned and half stained with H&E to detect alterations in morphology while others were stained with proliferating cell nuclear antigen (PCNA) to determine the number of cells expressing this protein.

Results:

The Sponsor's summary table of study results is reproduced below.

Effects of TAK-375 on melatonin and thyroid hormone levels and on histology of the Harderian glands in aged mice (Study No. 03-3757a)

Animals		B6C3F ₁ mouse, 62-63 weeks of age			
Test article		TAK-375			
Dosage level (mg/kg/day)		Control ^{a)}	1	30	300
No. of animals (M/F)		42 ^{b)} / 54 ^{b)}	42 ^{b)} / 54 ^{b)}	42 ^{b)} / 54 ^{b)}	42 ^{b)} / 54 ^{b)}
Mortality (M/F)		0 / 0	0 / 0	0 / 0	0 / 0
Clinical signs		-	-	-	-
Body weights		-	-	-	-
Plasma hormone levels (week 4, group mean values) (M/F)					
Melatonin (pg/mL)	1 h	1.82:2.39	2.32:2.53	↑7.14: ↑6.54	↑79.27: ↑48.72
	12-14 h	0.57:1.34	0.88:1.05	↑1.55:1.56	↑4.43: ↑2.20
TSH (ng/mL)	1 h	5.15:4.40	5.18:4.55	5.23:4.53	5.19:3.93
T3 (ng/dL)	1 h	190.8:81.5	92.9:84.2	113.8:80.1	101.8:80.6
T4 (µg/dL)	1 h	2.44:2.39	2.56:2.52	2.86:2.15	2.23:2.31
Harderian gland hormone levels (group mean values) (M/F)					
Melatonin ^{d)} (pg/100 mg of tissue)	1 h	5.5	5.5	↑0.439:0.266	↑5.327: ↑2.739
	12-14 h	5.5	5.5	5.5	5.5
Organ weight (Harderian gland)		-	-	-	-
Necropsy (Harderian glands)					
Nodule (M/F)		1/1	3/0	1/0	0/0
White foci (M/F)		0/2	0/0	0/0	1/0
Histopathology ^{e)} (Harderian glands)					
		-	-	-	-

- No treatment-related effects. M: Male, F: Female. ↑: Increase. T3: Triiodothyronine. T4: Thyroxine. TSH: Thyroid stimulating hormone. 1 h: About 1 hour after dosing. 12-14 h: About 12-14 hours after dosing. 5: Not calculated because 3/6 or more values were lower than the limit of quantification. a) 0.5 w/v% methylcellulose solution. b) 36 animals for determination of plasma or Harderian gland hormone levels and 6 animals for histopathology. c) 36 animals for determination of plasma or Harderian gland hormone levels. 6 animals for histopathology and remaining 12 animals were spare. for determination of plasma hormone levels. d) Means were calculated when 4/6 data were obtained in each group. e) Including PCNA immunohistochemistry.

No deaths were observed at any dose level during the course of the study. No unexpected and treatment-related clinical signs were observed. Body weight was unchanged with TAK-375 administration when compared with control animals. Assessment of plasma samples from rats revealed no significant effects of TAK-375 on plasma concentrations of thyroid hormone or TSH at any dose level. However, clear and dose-dependent increases in plasma melatonin levels were observed at doses of ≥ 30 mg/kg/day in both males and females at 1 hr post-TAK-375 administration and at 12-14 hr afterwards (though females in the 30 mg/kg/day group at 12-14 hr was not significantly increased). In male rats at 1 hr after TAK-375 administration, plasma melatonin concentrations were increased 3.9- and 43.6-fold over controls with 30 and 300 mg/kg/day dosing, respectively while in females these dose levels were associated with

increases of 2.5- and 20.4-fold. At 12-14 hr after TAK-375 this increase was less pronounced but was still significant with doses of 30 mg/kg/day and greater. Males in all cases were more sensitive to the effects of TAK-375 than females. Assessment of the Harderian gland from treated animals revealed that with 30 mg/kg/day or greater dose levels, also demonstrated a significant increase in Harderian gland melatonin. This did not reach statistical significance in females at 30 mg/kg/day but this may be a false negative as control values of 3/6 or more animals had values below the limit of quantitation and thus could not be determined. Harderian gland organ weight and histopathology, including staining for PCNA which detects cells in proliferation, was not different from control at any dose of TAK-375 administered.

Although the reasons for excluding the plasma samples and Harderian gland from some animals with white foci and nodules on the Harderian gland while including others was not made clear, no evidence of a difference in plasma hormone levels or Harderian gland melatonin levels were observed between animals which had these nodules and those that did not according to the Sponsor. Therefore the Sponsor believes the data is not biased in any direction on these measures.

Conclusions:

TAK-375 appears to alter the concentration of plasma melatonin levels as well as melatonin levels in the Harderian gland without affecting thyroid hormones. Additionally, no evidence was observed to suggest that TAK-375 has the potential to alter the structure or proliferative state of the Harderian gland with doses up to 300 mg/kg/day for 4 weeks in aged rats.

Study title: Effects of orally administered TAK-375 on plasma hormone levels in rats.

Group	Number of animals	LH (ng/mL)	FSH (ng/mL)	Testosterone (ng/mL)	Thyroxine (T4) (ug/dL)	Triiodothyronine (T3) (ng/mL)	FSH (ng/mL)
1M	10	2.58± 0.87	21.50± 4.86	4.11± 2.47	4.23± 0.99	1.29± 0.17	4.83± 2.81
2M	9 *	2.15± 0.37	17.64± 2.98	3.35± 2.02	4.47± 0.60	1.15± 0.12	2.78± 1.75
3M	10	2.06± 0.23	18.51± 2.74	3.96± 1.89	4.71± 0.37	1.18± 0.09	2.71± 1.44
4M	10	2.40± 0.37	17.71± 2.88	2.13± 1.39	5.22± 0.96	1.24± 0.12	2.74± 1.02
5M	10	2.18± 0.19	18.69± 3.96	2.37± 0.58**	4.49± 1.34	1.25± 0.14	3.44± 2.90

Group	Number of animals	ACTH (pg/mL)	Corticosterone (ng/mL)
1M	10	16.11 ± 20.7	42 ± 52
2M	9 *	1.81 ± 4.5	39 ± 51
3M	10	0.01 ± 0.0*	17 ± 26
4M	10	8.21 ± 27.4	14 ± 10
5M	10	7.71 ± 24.3	18 ± 12

* Significantly different from group 1 (p<0.05 or p<0.01)
 * : See text for the reason of decrease in the number of animals.

Study No: M-11-73
 Site and testing facility: Drug Safety Res Labs, Takeda Chemical Industries, Osaka, Japan.
 GLP compliance: NOT REPORTED
 QA- Reports Yes () No () NOT REPORTED
 Study initiation/completion: Jul 1998-Oct 1998
 Lot and batch numbers: M-375-004
 Protocol reviewed by Division Yes () No (x)
 Stability/purity: Not reported

Methods:

- Species/strain: Sprague-Dawley rats. Males, females and ovariectomized females.
- Age/wt: 11wk old males and 12wk old females/164-189g m and 130-151g f.
- Doses employed: 6, 20, 60, 200mg/kg/d.
- Route of Administration: oral gavage.
- Number of animals/sex/dosing group: 10/sex/dose; dose volume 10ml/kg; the control group received the vehicle control 0.5% MC. The drug was administered in the morning.

Comments:

This study was done due to the changes observed in some reproductive parameters in the repro studies and the thyroid changes and histopath changes in the adrenals in the 1month rat tox study.

Study Design:

TAK-375 was orally administered via gavage to 10/sex/dose rats once daily for 1wk at the following doses: 0, 6, 20, 60, and 200mg/kg/d. the following plasma hormone levels were determined 1hr post the last dose: LH, FSH, T4, T3m ACTH, and corticosterone; LH & FSH levels were measured in ovariectomized females and testosterone and prolactin levels were determined only in males and ovariectomized females.

Results:

The results were highly variable in all groups and the control therefore, a clear conclusion on some hormone levels could not be made. There was a significant decrease in testosterone levels and in T4 in the 200mg/kg male and female groups respectively. ACTH levels were decreased in all drug groups (significant only in 20mg/kg group males), relative to the control but were higher than the control in female rats where the control value was 0. Other changes were seen but no conclusion can be made because of the variation in the data (tables from sponsor).

In summary, the sponsor contributes the decrease in testosterone and T4 levels to the pharmacological effects of the drug being a melatonin agonist. Although this may be true, a drug adverse effect can not be ruled out since some of these changes were seen to correlate to some histopath and/or organ wt changes, or effects on reproductive parameters in previously conducted rat tox and reproductive studies.

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Effects of Orally Administered TAK-375 on Plasma Hormone Levels in Rats (2787/su)

Animal	CD(SD)IGS rats aged 11 or 12 weeks at the start of treatment					
Test article	Control ¹⁾	TAK-375				
Dose (mg/kg/day)	0	6	20	60	200	
No. of animals (M:F:ovariectomized F)	10:10:10	10:10:10	10:10:10	10:10:10	10:10:10	
Mortality (M:F:ovariectomized F)	0:0:0	0:0:0	0:0:0	0:0:0	0:0:0	
LH (ng/mL)	(M)	2.58	2.15	2.06	2.40	2.18
	(F) ²⁾	10.96	10.70	10.75	11.12	10.42
FSH (ng/mL)	(M)	21.50	17.64	18.51	17.71	18.69
	(F) ²⁾	52.33	50.72	54.35	51.06	50.71
Testosterone (ng/mL)	(M)	4.11	3.35	3.96	2.13	1.37 **
Prolactin (ng/mL)	(F) ²⁾	1.07	2.02	0.72	0.09	0.54
ACTH (pg/mL)	(M)	14.3	1.6	0 *	8.7	7.7
	(F)	0	2.8	5.3	5.0	10.2
Corticosterone (ng/mL)	(M)	47	39	17	14	16
	(F)	129	96	169	157	193
TSH (ng/mL)	(M)	4.83	2.78	2.71	2.74	3.44
	(F)	0.89	0.83	1.20	1.62	1.40
T ₄ (µg/dL)	(M)	4.23	4.47	4.71	5.22	4.49
	(F)	3.47	3.39	3.53	3.43	2.54 *
T ₃ (ng/mL)	(M)	1.29	1.15	1.18	1.24	1.25
	(F)	1.11	1.05	1.07	1.03	1.02
Conclusion	In the 200 mg/kg group, plasma testosterone and T ₄ concentrations were decreased in males and females, respectively.					

Mean values of 10 or 9 animals, M: Male, F: Female.

*, **: Significantly different from control (*: $p \leq 0.05$ or **: $p \leq 0.01$)

1): 0.5 w/v% methylcellulose solution, 2): Ovariectomized rats were used.

Study Title: Effects of TAK-375 on Plasma Estradiol and Prolactin Levels in Female Rats

Key findings:

- TAK-375 administered to female rats at doses of 0, 15, 60 or 250 mg/kg/day by oral gavage for 5 weeks **did not affect plasma concentrations of estradiol or prolactin.**
- TAK-375 at dose levels of ≥ 60 mg/kg/day was associated with decreased body weight and an **increase in the frequency of animals demonstrating irregular estrous cycles.** Irregularity was characterized by a prolongation of the cycle due to lengthening of time spent in metestrus.
- TAK-375 250 mg/kg/day was associated with a decrease in food consumption which likely is the causative factor in the decrease in body weight observed. No macroscopic alterations of organs were observed on necropsy with visual inspection.

Study #: M-11-00565

Vol/Page: N/A

Conducting laboratory and location: Drug Safety Research Laboratories, Takeda Chemical Industries, LTd. Yodogawa-ku, Osaka 532-8686, Japan

Date of study report: January 30, 2003

GLP compliance: No

QA report: No

Test article: TAK-375 (Lot # M375-026), purity [] %, stability validated based on Sponsor's previous assays with a different TAK-375 lot.

Methods: TAK-375 was administered by oral gavage to female Sprague-Dawley rats (12-weeks old) at doses of 0 (0.5 w/v% methylcellulose), 15, 60 or 250 mg/kg/day for approximately 5 weeks (34 days). Observations included clinical signs daily, body weight twice a week, food consumption determined weekly and averaged to a daily value. Vaginal smears were taken from all females 2 weeks prior to start of dosing and twice daily (morning and afternoon) during dosing with one smear on the last day of dosing and on the day of necropsy. Smears were stained with Giemsa's solution to determine estrous cycle phase. Estrous cycle phases were classified as proestrus, estrus, metestrus, or diestrus while estrous cycles were classified as being regular in cycle if they lasted 4 or 5 days, irregular if cycles deviated from 4 or 5 days, or no cycle if estrus phase was absent. Plasma estradiol and prolactin levels were measured during the last week of the dosing period at the time of proestrus and metestrus phases from each female. Plasma estradiol and prolactin levels were determined by means of radioimmunoassay and enzyme immunoassay methods at []

[] The day after the last dosing day animals were sacrificed and gross macroscopic inspection done on the internal organs with retention of uterus and ovaries which were fixed for histologic assessment.

Results: No animals died during the course of the study. The main treatment-related clinical sign observed was salivation with doses ≥ 60 mg/kg/day which the Sponsor attributes to the bitter taste of the drug. Reductions in body weight were observed over these same doses with food consumption reduced significantly only in the 250 mg/kg/day group. A dose-related increase in irregular estrus was observed in rats with administration of TAK-375 at ≥ 60 mg/kg/day (2, 0, 4, and 7 rats with irregular cycles associated with doses of 0, 15, 60, and 250 mg/kg/day). At dose levels of 60 mg/kg/day and greater the estrous cycles were prolonged when compared with control animals. This prolongation was associated with an increased duration of the metestrus phase. No effects were observed however on plasma estradiol or prolactin levels with dosing of TAK-375 up to 250 mg/kg/day for 5 weeks. Macroscopic examination of organs at necropsy revealed no apparent effect of TAK-375. The Sponsor's summary table is reproduced below.

Effects of TAK-375 on Plasma Estradiol and Prolactin Levels in Female Rats (Study No. 02-362/fe)

Animals	~:CD(SD)IGS rats, 10-week-old females			
Test article	Control	TAK-375		
Dosage level (mg/kg/day)	0	15	60	250
Dosage volume (mL/kg)	5	5	5	5
No. of animals	12	12	11 ^{a)}	12
No. of deaths	0	0	0	0
Clinical signs	-	-	Salivation	Salivation
Body weight change	-	-	↓#	↓#
Food consumption	-	-	-	↓#
Estrous cycles	-	-	Prolongation#	Prolongation#
Plasma hormone levels				
Estradiol (pg/mL) P	65.3±20.7	53.3±19.4	56.0±19.1	59.3±29.1
M	6.3±6.6	5.5±3.5	5.2±6.9	6.4±4.0
Prolactin (pg/mL) P	203.32±111.65	254.37±158.75	253.58±242.50	265.90±111.30
M	42.85±63.63	44.29±76.51	20.88±20.05	6.84±5.28
Necropsy findings	-	-	-	-
Conclusion	No significant changes in the plasma estradiol level or the plasma prolactin level were observed even at 250 mg/kg/day.			

Control: 0.5 w/v% methylcellulose solution, -: No treatment-related effects, #: Adverse effects

↓: Suppressed or decreased, NOAEL: No-observed-adverse-effect dosage level

P: Proestrus phase, M: Metestrus phase

a): One of the 12 females in this group died due to an intubation error.

Study Title: Effects of TAK-375 on plasma melatonin and dehydroepiandrosterone (DHEA) levels and on the histology of hepatocytes in male rats.

Key findings:

- Male Sprague-Dawley rats were administered TAK-375 by oral gavage at doses of 0, 15, 60, 250 or 1000 mg/kg/day for 4 weeks to assess potential effects on dehydroepiandrosterone levels and liver histopathology which might explain hepatic tumors observed in a 2-year rat bioassay.
- **Dose-dependent ↑melatonin** (12 – 52-fold) was observed with doses ≥ 60 mg/kg/day at 1 hr post-dosing and was maintained with doses ≥ 250 mg/kg/day (4 – 5-fold) at 15 hr after TAK-375 administration. ↑DHEA (2 – 3-fold) and corticosterone (approximately 2-fold) with TAK-375 doses ≥ 250 mg/kg/day. Liver weight (absolute and relative) was dose-dependently observed to increase with TAK-375 levels ≥ 250 mg/kg/day. Examination of liver tissue by light and electron microscopy demonstrated evidence of a **proliferation of smooth endoplasmic reticulum without evidence of peroxisome proliferation** at a dose of 1000 mg/kg/day.
- Evidence of hepatic cell proliferation, as measured by proliferation cell nuclear antigen (PCNA), was not observed with TAK-375 at any level when compared with controls but this may be due to unusually high levels observed in two control animals. With comparison of TAK-375 dose groups, an elevation in PCNA labeling appeared to occur at the 1000 mg/kg/day level.
- Therefore the hypothesis that hepatic tumors observed in rat 2-year bioassay are related to peroxisome proliferation induced by elevated DHEA levels caused by TAK-375 administration is not supported. Instead, the observation of smooth ER proliferation is consistent with a hepatic enzyme induction mechanism underlying the tumor formation.

Study #: M-11-00599

Vol/Page: N/A

Conducting laboratory and location: ㄥ ㄑ Drug Safety Research Center, Takeda Chemical Industries, Ltd. Yamaguchi 743-8502, Japan

Date of study report: April 25, 2003

GLP compliance: No

QA report: No

Test article: TAK-375 (Lot # M375-026), purity ㄥ ㄑ, stability supported by test of .01 to 10 g/dL in an air-tight ㄣ bottle after ㄠ at 5°C and 6 hr at 25°C (studies in Appendix)

Methods: Male Sprague-Dawley rats aged 6 weeks at the initiation of dosing period were administered TAK-375 at doses of 0 (0.5 w/v% methylcellulose), 15, 60, 250 or 1000 mg/kg/day by oral gavage daily for 4 weeks. Observed parameters included clinical signs and mortality, body weights, urine sampling (for use in another study), plasma hormone levels of melatonin (at 1 hr and 15 hr post-TAK-375 administration on final day), DHEA (at 1 hr and 8 hr post-TAK-375 administration on final day) and corticosterone, gross pathology and liver weight, and liver histopathology including immunostaining for proliferating cell nuclear antigen (PCNA), a marker for cells undergoing DNA replication or repair. Liver tissue was also prepared for electron microscopy and samples of liver were used for assessment of hepatic acyl-CoA oxidase, a marker enzyme for peroxisome proliferation.

Results: As can be seen in the summary table provided by the Sponsor below, TAK-375 administration of doses up to 1000 mg/kg/day for 4 weeks in rats was not associated with mortality and clinical signs were minor and limited to increased salivation immediately after dosing with ≥ 250 mg/kg/day and CNS depressant effects at the high dose of 1000 mg/kg/day. Body weight remained unchanged with dosing ≤ 250 mg/kg/day though a decrease was observed at the high dose. As has been observed in a previous study in aged rats (M-11-00709), plasma melatonin levels exhibited a dose-dependent increase when measured both 1 hr and 15 hr after the final administration of TAK-375. Increases in melatonin were significant at doses ≥ 60 mg/kg/day at the 1 hr timepoint and ≥ 250 mg/kg/day when measured at 15 hr after the final dose. These changes were quite large relative to control animal melatonin concentrations (12-, 19- and 50-fold over baseline at 1hr with dosing ≥ 60 mg/kg/day; 3.7 and 5-fold over baseline at 15 hr with dosing ≥ 250 mg/kg/day). Levels of circulating DHEA were also dose-dependently increased with doses ≥ 250 mg/kg/day with a maximal increase of 3.2-fold over control when measured 1 hr post-administration of TAK-375. At 8 hr this increase was only significant in the 1000 mg/kg/day group with a maximal increase of 4-fold over control animals. Corticosterone levels were increased in animals treated with 250 mg/kg/day or higher dose of TAK-375 when measured 8 hr after the last administration of test article. On gross pathology, the liver appeared enlarged and was increased in weight with doses of 1000 mg/kg/day though liver weight was increased relative to body weight with doses ≥ 250 mg/kg/day. Hepatocyte immunohistochemistry with PCNA revealed a non-significant increase in this cell proliferation marker though Sponsor notes that this may be due to an elevated level in 2 control animals which may obscure a true effect. A comparison of treated animals would suggest a significant elevation in PCNA (~ 4-fold) at the highest dose. Electron microscopic examination of liver tissue revealed an increase in smooth endoplasmic reticulum consistent with the Sponsor's observation of induced hepatic metabolism. No evidence of an increase in acyl-CoA levels, which would be indicative of peroxisome proliferation, was observed.

Animal	CD(SD)IGS Rats, 6 weeks of age					
Test article	TAK-375					
Dose (mg/kg/day)	Control ¹⁾	15	60	250	1000	
Dosage vol. (mL/kg/day)	5	5	5	5	5	
No. of animals (M)	10	10	10	10	13 ²⁾	
Mortality (M)	0	0	1 ³⁾	0	1 ³⁾	
Clinical signs	—	—	—	Salivation Colored urine, Decreased locomotor activity, Ataxic gait, Bradypnea		
Body weight	—	—	—	—	↓	
Plasma hormone levels (week 4, group mean values)						
Melatonin (pg/mL)	1 h ⁴⁾	3.91	7.99	↑47.42	↑75.79	↑201.63
	15 h ⁵⁾	42.35	27.81	89.08	↑154.81	↑211.65
DHEA (ng/mL)	1 h ⁴⁾	0.09	0.09	0.09	↑0.17	↑0.29
	8 h ⁶⁾	0.07	0.14	0.15	0.13	↑0.28
Corticosterone (ng/ml)	8 h ⁶⁾	133.5	140.7	190.5	↑240.5	↑259.5
Gross pathology	—	—	—	—	Liver: large	
Organ weights (Liver)	—	—	—	Towards ↑	↑	
PCNA labeling index (Liver) ⁷⁾	7.2	3.3	3.2	3.4	12.4	
Electron microscopy (Liver) ⁸⁾	—	/	/	/	Proliferation of sER	
Acyl-CoA oxidase activity ⁷⁾ (Liver, U/g protein)	2.43	2.18	2.34	↓1.99	↓1.68	

M: Male, 1) Vehicle: 0.5 w/v% methylcellulose solution, 2): 10 of 13 animals were used for gross pathology and determinations of organ weights, PCNA labeling index and plasma hormone concentrations, 3): Accidental death due to excess hemorrhage when sampling blood for hormone assay, 4): 1 hour after dosing (around 10:00), 5): 13-15 hours after dosing (23:00-1:00), 6): 7-8 hours after dosing (16:00-17:00), 7): Group mean values, 8): N=3, —: No abnormality, ↑: Increase, ↓: Decrease, PCNA: Proliferating cell nuclear antigen, sER: Smooth endoplasmic reticulum, DHEA: Dehydroepiandrosterone.

Conclusions:

Results from this study do not support a mechanism offered by the Sponsor which suggested the hepatic tumors observed in the 2-year rat bioassay may be due the induction of peroxisome proliferation as a result of TAK-375-induced elevation of plasma DHEA levels. Although DHEA is clearly elevated in rats administered TAK-375 for 4 weeks, the microscopic evaluation of hepatic tissue does not reveal evidence of peroxisome proliferation, instead demonstrating ultrastructural evidence consistent with hepatic enzyme induction which is a known effect of TAK-375. It is possible, however, that a longer exposure to TAK-375 may be necessary to initiate peroxisome proliferation though it would be expected that evidence could be seen with the duration of exposure used in the present study.

Study Title: Effects of 7-day Treatment with TAK-375 on Plasma Testosterone and Leutenizing Hormone Levels in Male Rats

Study purpose: This study was undertaken to investigate a potential mechanistic explanation for the Leydig cell tumors observed in the rat with doses of ≥ 250 mg/kg/day in a 2-year carcinogenicity bioassay. Leydig cell tumor development has been linked to reductions in circulating testosterone levels with consequent elevation in lutenizing hormone (LH) released from the pituitary and hyperstimulation of these LH-sensitive cells.

Key findings:

- Administration of TAK-375 by oral gavage in doses of 0, 250 or 1000 mg/kg/day for 7 days was associated with a significant reduction in circulating testosterone levels with both 250 and 1000 mg/kg/day doses lasting 3 hr and 5 hr, respectively. No alteration in circulating LH levels was associated with this exposure to TAK-375, however.

Study #: M-11-00689**Vol/Page:** N/A**Conducting laboratory and location:** [Drug Safety Research Center, Takeda Chemical Industries, Ltd., Yamaguchi 743-8502, Japan**Date of study report:** May 8, 2004**GLP compliance:** No**QA report:** No**Test article:** TAK-375 (Lot M375-030), purity [% with COA and stability report contained in Appendix**Methods:** Male Sprague-Dawley rats (n=30/group; 40 for HD), 11-weeks old at initiation of dosing, were administered TAK-375 at doses of 0 (0.5 w/v% methylcellulose), 250 or 1000 mg/kg/day by oral gavage for 7 days. Clinical signs, mortality, body weight and a macroscopic examination of visceral organs were recorded. Plasma was obtained from trunk blood upon decapitation and was taken 1, 3, 5, or 8 hr after the last dose was administered. Plasma concentrations of LH and testosterone were determined using radioimmunoassay methods at a contract laboratory, []

Results: No mortality was observed in the control or TAK-375 200 mg/kg/day dose group. Mortality was observed at 1000 mg/kg/day (10/50 animals died) and was characterized pre-death by prone position, lateral recumbency, bradypnea, hypothermia, reduced feces, and reduced locomotor activity. These findings, as well as mortality, were principally observed over the first few days of dosing with some apparent tolerance acquired over the last few days. The 250 mg/kg/day group demonstrated reduced activity as well but did not display the other aforementioned clinical signs. Body weight was significantly decreased in the 1000 mg/kg/day group. Food consumption was not recorded to assess whether this was responsible for the observed changes in weight though this would seem likely. Gross inspection of the organs at necropsy did not reveal a treatment-related effect. Assessment of plasma hormone levels indicated that TAK-375 did appear to reduce circulating testosterone levels with a dose of 250 and 1000 mg/kg/day and lasting approximately 3 hr and 5 hr, respectively. TAK-375 at 250 mg/kg/day for 7 days appeared to decrease testosterone levels by 60-70% compared with control animals for the first 3 hrs after dosing while TAK-375 given at 1000 mg/kg/day for the same duration decreased testosterone levels by 80-85% for the first 3 hrs after dosing and at 5 hr after drug administration was still reduced by 60% of control animal values. At the 8 hr timepoint there was no difference with either dose level when compared with controls on levels of testosterone. In contrast, LH levels were unaffected by TAK-375 treatment up to 1000 mg/kg/day for 7 days at all timepoints measured. See Sponsor's table which was reproduced below.

Effects of 7-day Treatment with TAK-375 on Plasma Testosterone and Luteinizing Hormone Levels in Male Rats (03-489/fe)

Species/strain/age	Rats \ CD(SD)IGS/11-week-old males			
Treatment	7 days, Orally by gavage			
Test article	Control ^{a)}	TAK-375		
Dosage level (mg/kg/day)	0	250	1000	
Dosage volume (mL/kg/day)	10	10	10	
No. of animals	40	40	50	
No. of deaths	0	0	10	
Clinical signs	-	↓ Locomotor activity	Prone position Lateral position, Bradypnea Hypothermia, Soiled fur ↓ Feces, ↓ Locomotor activity	
Body weight gain	-	-	↓	
Necropsy findings	-	-	-	
Plasma hormone Levels (Mean±S.D., n=10)				
Testosterone (ng/mL)	1 hour	5.40±2.39	1.79±0.87 ^w	0.79±0.53 ^w
	3 hour	2.05±0.92	0.81±0.63 ^w	0.39±0.26 ^w
	5 hour	1.55±0.87	1.79±1.17	0.63±0.30 ^w
	8 hour	1.01±0.55	1.24±0.94	1.46±1.07
LH (ng/mL)	1 hour	1.97±0.89	1.98±0.50	1.77±0.38
	3 hour	1.59±0.43	2.08±0.41	1.76±0.59
	5 hour	2.09±0.36	2.15±0.46	2.07±0.39
	8 hour	1.70±0.67	1.71±0.83	1.75±0.39
Conclusion	The plasma testosterone level decreased but plasma LH levels did not change under the present experimental conditions			

a). 0.5 w/v% methylcellulose solution. ↓: Decreased/Suppressed. LH: Luteinizing hormone
w. Significantly different from the control group (p≤0.05, Shirley-Williams' test)

Conclusions: Oral administration of TAK-375 to rats with doses of ≥ 250 mg/kg/day for 7 days produced a dose-dependent decrease in circulating testosterone concentrations which was observed up to 5 hr after drug administration. Administration of TAK-375 did not alter the levels of circulating LH concentrations, however, over this timeframe. This study does not directly support a LH-induced mechanism for the Leydig cell tumors as proposed by the Sponsor though the latency to LH alterations in the face of a decrease in prevailing testosterone concentrations in rats is not discussed.

Study Title: Effects of 7-day Treatment with TAK-375 on Plasma Testosterone and Luteinizing Hormone Levels in Male Rats – Supplemental Study

Study purpose: This study was undertaken to support a potential mechanistic explanation for the Leydig cell tumor development observed in the 2-year rat bioassay. The previous study of TAK-375 on plasma testosterone and luteinizing (LH) hormone concentrations revealed a reduction of testosterone but no change in LH with administration of TAK-375 for 1 week and sampling times at 1, 3, 5, and 8 hr after the final dose. The pulsatile release of LH is reported to be 30 minutes in rats and the Sponsor believes they may have missed effects on LH due to the 2-3 hr between time-points. The present study uses sampling at interim time-points of 2, 4, and 6 hr after the final dose to fill out the 8 hr interval and pick up effects potentially missed in the first study.

Key findings:

- Oral administration of TAK-375 to male rats at doses of 250 and 1000 mg/kg/day depress testosterone concentrations in plasma significantly for 4 hr after the final dose but no effect of TAK-375 is observed on plasma LH concentration up to 6 hr after drug administration.

Study #: M-11-00690

Vol/Page: N/A

Conducting laboratory and location: T Drug Safety Research Center, Takeda Chemical Industries, Ltd., Yamaguchi 743-8502, Japan

Date of study report: May 8, 2004

GLP compliance: No

QA report: No

Test article: TAK-375, lot # M375-030, purity T J%, stability confirmation documented in Appendix

Methods: As in previous study though with different plasma sampling time-points. Briefly, male Sprague-Dawley rats (n = 30; 40 for HD), 11-weeks old at initiation of dosing, were administered TAK-375 at doses of 0 (0.5 w/v% methylcellulose), 250 or 1000 mg/kg/day by oral gavage for 7 days. Clinical signs, mortality, body weight and a macroscopic examination of visceral organs were recorded. Plasma was obtained from trunk blood upon decapitation and was taken 2, 4, or 6 hr after the last dose was administered. Plasma concentrations of LH and testosterone were determined using radioimmunoassay methods at a contract laboratory, T J

Results: Clinical signs included reduced spontaneous locomotor activity in LD animals with more significant sedation at the HD including altered position, reduced respiratory rate and a reduction in fecal production. Body weight was significantly reduced only in the HD group. Four animals in the HD group died during the treatment period of the study, no deaths in the LD group. Necropsy revealed no gross organ findings. Results of the plasma hormone analysis indicated that, as in the previous study, a significant decrease in testosterone was associated with TAK-375 $\geq$ 250 mg/kg/day for up to 4 hr after the last TAK-375 administration with recovery by the 6 hr time-point. As in the previous study, no effect on plasma LH was revealed at any time-point measured. See Sponsor's tabulated summary of results reproduced below.

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Effects of 7-day Treatment with TAK-375 on Plasma Testosterone and Luteinizing Hormone Levels in Male Rats - Supplemental study - (33-699/fe)

Species/strain/age	Rats / D(SD)IGS/11-week-old males			
Treatment	7 days, Orally by gavage			
Test article	Control ^a	TAK-375		
Dosage level (mg/kg/day)	0	250	1000	
Dosage volume (mL/kg/day)	10	10	10	
No. of animals	30	30	40	
No. of deaths	0	0	4	
Clinical signs	-	↓Locomotor activity	Prone position Lateral position, Bradypnea ↓Feces, ↓Locomotor activity	
Body weight gain	-	-	↓	
Necropsy findings	-	-	-	
Plasma hormone Levels (Mean±S.D., n=10)				
Testosterone (ng/mL)	2 hour	2.98±1.19	0.83±0.92 ^w	0.35±0.25 ^w
	4 hour	1.00±0.61	0.41±0.19 ^w	0.28±0.18 ^w
	6 hour	1.08±0.59	1.08±0.96	1.16±1.24
LH (ng/mL)	2 hour	1.62±0.50	1.66±0.33	1.55±0.26
	4 hour	1.73±0.45	1.80±0.57	1.63±0.27
	6 hour	1.73±0.31	2.01±0.44	1.95±0.40
Conclusion	The plasma testosterone level decreased but the plasma LH level did not change under the present experimental conditions.			

a) 0.5 w/v% methylcellulose solution, - : No treatment-related effects, ↓: Decreased/Suppressed LH: Luteinizing hormone
w: Significantly different from the control group (p≤0.05, Shirley-Williams' test)

Conclusions: Under the conditions of this study and when combined with the previous study, no apparent effect of TAK-375 can be seen on plasma LH levels for up to 8 hrs after the final TAK-375 administration in a 1-week repeated dose study. A significant but transient depression of testosterone is observed to occur with oral administration of TAK-375 which resolves within 6 hr.

Study Title: Effects of 4-week Treatment with TAK-375 on Plasma Testosterone and Luteinizing Hormone Levels in Male Rats

Study purpose: This study was undertaken to support a potential mechanistic explanation for the Leydig cell tumor development observed in the 2-year rat bioassay. Previous 1-week repeat-dose studies with TAK-375 on plasma testosterone and LH concentrations in male rats revealed a transient suppression of testosterone but did not detect any change in LH concentrations. The present study was designed to produce longer-term exposure to TAK-375 in male rats due to a cited report which suggested that increases in plasma LH in the rat due to reduced circulating testosterone require 2 weeks of testosterone suppression to develop.

Key findings:

- Oral administration of TAK-375 at doses of 0 (0.5 w/v% methylcellulose), 250 or 1000 mg/kg/day for 4-weeks to male rats produced transient suppression of plasma testosterone levels in rats treated with 250 and 1000 mg/kg/day TAK-375 at a Week 2 interim analysis and at Week 4 and an increase in plasma LH concentrations observed only in 1000 mg/kg/day group rats at 4.5 hr and 24 hr after the final dose on Week 4.

Study #: M-11-00691

Vol/Page: N/A

Conducting laboratory and location: [] Drug Safety Research Center, Takeda Chemical Industries, Ltd. Yamaguchi 743-8502, Japan

Date of study report: May 31, 2004

GLP compliance: No

QA report: No

Test article: TAK-375, Lot # M375-030, purity [] 100%, stability confirmation documented in Appendix

Methods: Generally as described in previous studies with the exception of 4-week rather than 1-week repeated dosing. Briefly, male Sprague-Dawley rats (n = 50; 60 for HD), 8-weeks old at initiation of dosing, were administered TAK-375 at doses of 0 (0.5 w/v% methylcellulose), 250 or 1000 mg/kg/day by oral gavage for 28 days. Clinical signs, mortality, and body weight were recorded. Gross necropsy with macroscopic assessment and organ weights were not conducted. Plasma was obtained from the jugular vein at 3, 4, 5, and 8 hr after dosing at Week 2 and at 3, 3.5, 4, 4.5, 5, 8, 10 and 24 hr at Week 4 (after the 28th or 29th dose). Plasma concentrations of LH and testosterone were determined using radioimmunoassay methods at a contract laboratory. []

Results: All animals in the vehicle and 250 mg/kg/day TAK-375 group survived and demonstrated no clinical signs of note. One of 50 animals at 1000 mg/kg/day died during the treatment period (Day unknown), reason not determined. Clinical signs observed in surviving 1000 mg/kg/day dose males only included reduction in spontaneous locomotor activity and fecal droppings. Salivation observed is thought to be due to the bitterness of the test article. As in previous studies with shorter duration, a reduction in body weight was observed with the 1000 mg/kg/day dose level. Analysis of plasma obtained at the 2 week time-point revealed a significant though transient reduction in testosterone levels with TAK-375 concentrations of 250 mg/kg/day or 1000 mg/kg/day with this suppression lasting up to 4 hr after the last 1000 mg/kg/day TAK-375 dose. No alterations in plasma LH levels were observed up to 8 hr after the last dose. Analysis of plasma obtained after the 28th or 29th dose in Week 4 revealed a similar reduction in plasma testosterone levels with a similar temporal response but a significant increase in plasma LH was observed at 4.5 hr (1000 mg/kg/day group only) and 24 hr after the final 1000 mg/kg/day TAK-375 dose (250 and 1000 mg/kg/day group animals). Plasma LH appeared to be increased at the 8 hr time-point in HD animals as well, though this did not reach statistical significance. Plasma LH was not different in TAK-375-treated when compared to vehicle-treated rats at any other time-points. See the Sponsor's summary tables of results for the Week 2 and Week 4 assessments reproduced below.

Effects of 4-week Treatment with TAK-375 on Plasma Testosterone and Luteinizing Hormone Levels in Male Rats (04-066/fe) (1/2)

Species/strain/age	Rats \ CD(SD)IGS/8-week-old, males			
Treatment	4 weeks, Orally by gavage			
Test article	Control ^{a)}	TAK-375		
Dosage level (mg/kg/day)	0	250	1000	
Dosage volume (mL/kg/day)	10	10	10	
No. of animals	40	40	50	
No. of deaths	0	0	1	
Clinical signs	-	-	Surviving males Salivation, ↓Feeces, ↓Locomotor activity Dead male Prone position, Lateral position, Soiled fur, ↓Feeces ↓Locomotor activity	
Body weight gain	-	-	↓	
Plasma hormone levels (Mean±S.D., n=10)				
Week 2				
Testosterone (ng/mL)	3 hours	1.29±0.90	0.36±0.25w	0.17±0.07w
	4 hours	1.36±1.31	0.55±0.43	0.20±0.15w
	5 hours	1.44±0.95	1.08±0.80	1.07±1.37
	8 hours	1.06±0.55	1.40±0.57	0.92±0.60
LH (ng/mL)	3 hours	1.40±0.78	1.27±0.76	1.50±0.63
	4 hours	1.33±0.75	1.23±0.73	1.25±0.77
	5 hours	0.85±0.68	1.26±0.19	1.29±0.75
	8 hours	0.77±0.69	1.04±0.64	1.16±0.75

a) 0.5 w/v% methylcellulose solution. - : No treatment-related effects. ↓: Decreased/Suppressed LH, Luteinizing hormone, w: Significantly different from the control group (p≤0.05, Williams' or Shirley-Williams' test)

Effects of 4-week Treatment with TAK-375 on Plasma Testosterone and Luteinizing Hormone Levels in Male Rats (04-066/fe) (2/2)

Test article	Control ^{a)}	TAK-375		
Dosage level (mg/kg/day)	0	250	1000	
Plasma hormone levels (Mean±S.D., n=10)				
Week 4				
Testosterone (ng/mL)	3 hours	1.99±1.18	0.39±0.39w	0.28±0.10w
	3.5 hours	2.00±1.29	0.36±0.28w	0.26±0.11w
	4 hours	1.65±1.23	0.67±0.39w	0.32±0.17w
	4.5 hours	1.48±0.95	0.94±0.84	0.28±0.30w
	5 hours	1.57±0.93	1.26±0.77	0.72±0.40w
	8 hours	1.01±0.51	1.69±1.91	0.85±0.70
	10 hours	0.94±0.37	1.60±1.10	1.29±0.90
	24 hours	4.17±1.82	3.36±1.42	4.49±2.83
LH (ng/mL)	3 hours	1.32±0.56	1.69±0.51	1.57±0.77
	3.5 hours	1.34±0.76	1.61±0.72	1.62±0.73
	4 hours	1.49±0.73	2.02±0.50	1.76±0.51
	4.5 hours	1.04±0.68	1.46±0.60	1.69±0.55w
	5 hours	1.40±0.81	1.63±0.73	1.47±0.25
	8 hours	1.42±0.63	1.76±0.40	2.00±0.88
	10 hours	1.29±0.61	1.47±0.63	1.34±0.87
	24 hours	1.05±0.77	1.86±0.51w	1.67±0.25w
Conclusion	The plasma testosterone level decreased, and the plasma LH level increased under the present experimental conditions			

a) 0.5 w/v% methylcellulose solution, LH Luteinizing hormone
w: Significantly different from the control group (p≤0.05, Williams' or Shirley-Williams' test)

Conclusions: Under the conditions of this study, a 4-week oral administration of TAK-375 to male rats produced transient suppression of plasma testosterone concentrations for up to 4 hr with doses ≥ 250 mg/kg/day while LH concentrations were significantly increased at 4.5 and 24 hr after the final 1000 mg/kg/day dose only. As the final time-point was 24 hr post drug administration demonstrated a significant increase in plasma LH concentrations and the plasma analysis time-point preceding this was 10 hr (at which time there was no difference between TAK-375-treated groups and controls), it is conceivable that a sustained increase between 10 hr and 24 hr may have been missed with this study protocol. It should be noted that the first time-point after drug administration measured is 3 hr post-drug, at which time no change in LH was noted which suggests any increase in plasma LH observed at 24 hr from the previous day dose did not last more than 3 hr. Although possible in the opinion of this reviewer it is difficult to imagine that transient and long-latency elevations of plasma LH concentrations would be responsible for the Leydig cell tumor development observed in the 2-year rat bioassay.

Impurity Studies

Several general toxicology and genetic toxicology studies were conducted for qualification of the potential drug substance impurities []

— (unknown impurity)

Study M-11-00098 Oral Single Dose Toxicity Study of TAK-375 Containing New Related Substance []

[] in Rats

Study M-11-00052 Bacterial Reversion Assay with TAK-375 (Lot No. M375-010)

[]

Study M-11-00356.001 Four Week Oral Gavage Toxicity Study of TAK-375 (Lot No. M375-017) in Rats

Study M-11-00353 Bacterial Reversion Assay with TAK-375 (Lot No. M375-017)

Study M-11-00354 Cytogenetic Assay with TAK-375 (Lot No. M375-017) in Chinese Hamster Lung (CHL) Cells

These studies were not formally reviewed as the specifications for TAK-375 indicate that the [] impurity will be kept below ICH threshold for qualification ([] %) and the [] impurity has been reduced ([]) such that no specification is necessary. Nevertheless, the acute toxicity study in rats with a TAK-375 lot (Lot #M375-010) containing the [] impurity at [] % revealed no differences in toxicity when compared with the same dose (2000 mg/kg) of TAK-375 utilizing a lot without this impurity (Lot #M375-022) indicating no special acute toxicities could be associated with the [] impurity. Additionally, the results of an Ames assay with the [] containing Lot #M375-010 was not mutagenic under experimental conditions. The [] impurity which was contained in a lot (Lot #M375-017) at [] also demonstrated no unique toxicities in the 4-week oral toxicity study in the rat as compared to a TAK-375 lot without this impurity and was negative for mutagenicity or clastogenicity in both the Bacterial Reversion (Ames) assay and Chromosomal Aberration assay using Chinese Hamster Lung Cells, respectively.

2.6.6.9 Discussion and Conclusions

General Toxicology Studies

Single-dose toxicology studies suggest TAK-375 has a relatively low order of acute oral toxicity with no mortality in monkeys administered 2000 mg/kg p.o. while in rats this same dose level did not produce mortality in males though in one female administered 2000 mg/kg/day died showing signs of TAK-375 intoxication including bradypnea, lacrimation, twitching and lying on one side. The high degree of first pass metabolism may certainly play a role in the absence of death, especially in monkeys which also do not produce the active metabolite M-II in significant concentrations. Major toxicities in rats that survived oral administration of TAK-375 at 2000 mg/kg dose were those indicating CNS toxicity and intoxication and showed full recovery. Monkeys given 2000 mg/kg acutely also demonstrated CNS toxicity characterized primarily by emesis though reductions in body temperature and reductions in heart rate were seen at all doses tested. These acute effects in both rat and monkey appear to be an exaggeration of melatonin receptor agonist pharmacology.

Repeat-dose toxicology studies conducted in mice, rats and monkeys revealed several areas of overlapping or consistent toxicity:

Central Nervous System

Signs of CNS depression or toxicity were observed in all nonclinical species tested and primarily consisted of decreased spontaneous locomotor activity with additional findings of mild respiratory depression in mice which was similar to rats in acute toxicity studies, salivation in rats given TAK-375, and emesis in monkeys. CNS depression may be expected of this compound as an extension or exaggeration of the pharmacologic effect as a sleep-promoting drug. Recovery was noted within hours of administration of the first dose generally and tolerance to the CNS effects appeared to occur in rodents within days though salivation in rats and was unaffected by repeated dosing and in monkeys tolerance was not observed to the emetic response which suggest a different mechanism. Salivation in rat studies is believed by the Sponsor to be due to the bitterness of the test article and is not a pharmacologic effect of the drug though evidence for this contention was not provided. Studies of the emetic properties of TAK-375 in monkeys demonstrated this effect was a direct effect of TAK-375 as a melatonin receptor agonist as luzindole, a specific ML_1 receptor antagonist, was capable of blocking this response as was the 5-HT₃ receptor antagonist granisetron suggesting the serotonergic system is involved in this circuit as well. The convulsions observed in 2 male monkeys in the 39-week chronic toxicity study occurred in the 3rd month of dosing in one monkey and 5th month in the other monkey and were not seen to recur with 6 and 4 months, respectively, of continued TAK-375 administration. Therefore, the toxicologic significance of this observation is unclear but appears likely to be incidental.

Liver

Increased liver weight was noted in the gross necropsy findings of all animal species used for general toxicology studies. In rodents, the increased liver weight was reflected in gross observations of enlargement and darkened character (the latter primarily in female mice and rats). AST and ALT were elevated in mice but not in rats in general and these elevations were < 2-fold. Total cholesterol and triglycerides were observed in rat toxicology studies but

elevations were < 2-fold and demonstrated recovery with cessation of dosing. Histologic examination of tissues from the liver revealed a consistent finding of centrilobular hepatocellular hypertrophy throughout the rodent studies. These findings, from the gross to the microscopic level, are typical of hepatic enzyme induction which may be considered to be an adaptive response to repeated exposure to a xenobiotic. Further support for this mechanistic explanation can be found in the reduced exposure to TAK-375 observed in the repeat-dose rodent toxicokinetic data as well as the direct assessments of hepatic enzyme activity in both general repeat-dose toxicology studies and dedicated *in vivo* metabolism studies which examined the potential for hepatic enzyme induction in mice and rats. Both rat and mouse toxicology studies detected significant increases in drug-metabolizing enzyme activity using the broad-spectrum P450 probe aminopyrine N-demethylase. Mice did not exhibit an induction of CYP2E1 as measured by the substrate aniline hydroxylase though this was significantly induced in the rat. Specific study of the characteristics of hepatic enzyme induction in rat and mice indicated TAK-375 is a potent and relatively broad drug-metabolizing enzyme inducer on par with phenobarbital with elevation of both Cytochrome b₅ and Cytochrome P450 contents. Thus the hepatic findings in rodents would appear to be adaptive or toxicologically benign. Studies in monkey, however, do reveal an effect on the liver which may not be explained by an adaptive change in metabolism. Evidence of increased liver weight was observed in both 4-week and the 39-week chronic toxicity study in the cynomolgus monkey without macroscopic evidence of abnormality. Microscopic examination of the liver from monkeys treated for 4-weeks did not reveal changes to the tissue. Assessment of aminopyrine N-demethylase and aniline hydroxylase activity from this 4-week treatment indicated some increase in global enzyme activity in females though not in males as a group and there was no evidence of a significant reduction in plasma exposure to TAK-375 over the course of the study. In the 39-week chronic toxicity study, no evidence of enzyme induction was observed using the same dose levels. Hepatocellular hypertrophy noted by the reviewing pathologist in the microscopic exam noted characterized the hypertrophy as non-centrilobular, non-zonal and diffuse. Furthermore, the affected cells were described as having pale cytoplasm with fine vesiculation. Taken together, this does not represent the distributional pattern or microscopic cellular characteristics expected to be observed with significant hepatic enzyme induction. In keeping with this lack of histologic evidence, exposure to TAK-375 was not reduced in this study with repeated administration of drug. Instead, a significant increase in ALT was demonstrated which in males was greater than 4-fold higher than control animals and became more wide-spread in the high-dose animals and severe over time. The lack of evidence for microsomal enzyme induction combined with increased liver weight, ALT enzyme elevation, and a dose-dependent increase in triglycerides suggests a more directly hepatotoxic profile of TAK-375 in the nonhuman primate. This hepatotoxicity is, however, likely to be due to TAK-375 itself and not the active M-II metabolite as this species is not observed in high concentrations in the monkey. Indeed, when M-II was administered intravenously for 4 weeks in a separate study there was no toxicity observed with high concentrations of M-II in circulation.

Thyroid gland

Toxicologic observations in the thyroid of nonclinical species were limited to increased thyroid weight with histologic evidence of follicular cell hypertrophy in the rat only. Thyroid hormones were altered in the rat with repeated administration of TAK-375 in all studies of 4-

weeks duration or greater. Changes typically consisted of a depression of T₃ and elevation in TSH, the latter hormone up to 3 – 8-fold control values in a 13-week study in the rat. Alterations in serum T₄ were not observed in any study, however. The Sponsor demonstrated an increase in UDP-glucuronosyltransferase activity involved in T₄ metabolism which supports a documented cascade of microsomal enzyme induction producing increased glucuronidation and subsequent excretion of T₄ which causes a transient reduction in circulating T₄ levels which removes feedback inhibition on TSH release from the pituitary and the increased TSH levels lead to both restores the proper level of circulating thyroid hormone level (principally T₄) and follicular cell hypertrophy. If this increase in microsomal enzyme activity is long term the excessive stimulation of the thyroid by TSH is believed to cause thyroid tumors (Vriend, 1983; Hood et al., 1999; Hood et al., 2003). Though several elements of this cascade generally seem in play (i.e. microsomal enzyme activation involving metabolism of a thyroid hormone, increased TSH levels, etc.) the levels of T₃ are observed to be reduced in circulation and the Sponsor has not demonstrated that the hepatic enzymes involved in T₃ glucuronidation and elimination are elevated in activity. There are several published studies which have demonstrated that induction of microsomal enzymes causing a reduction in T₄ and compensatory TSH increase without evidence of a change in T₃ levels such as was noted in the current studies (Liu et al., 1995; Hood et al., 2003). A reduced serum T₃ without a similar change in T₄ and consequent increase in TSH production could be effected by interference with thyroid hormone metabolism, specifically the 5'-monodeiodinase which is responsible for the conversion of T₄ → T₃ (Capen, 1997). Melatonin has recently been suggested to inhibit thyroid function through its anti-oxidant properties; reactive oxygen species and free radicals such as H₂O₂ are involved in thyroid hormone production (Karbownik and Lewinski, 2003) and therefore a direct effect of a melatonin receptor agonist such as TAK-375 on thyroid function cannot be ruled out.

No evidence for thyroid changes was observed in the mouse or monkey on gross or microscopic examination of this tissue, however. Although hepatic enzyme induction in the monkey was limited at best, significant microsomal induction was observed in the mouse which leads this reviewer to question whether this finding may also then be specific to the rat. With the present level of information the toxicologic significance of this finding in the rat for the determination of human risk is not clear though in rats the increased thyroid weight resolved with a 13-week treatment-free period suggesting the disruption produced is not permanent.

Adrenal gland

Studies in rodents consistently detected the development of adrenocortical vacuolization characterized as slight to mild in severity coincident with increased adrenal gland weight. This histopathologic change was observed to begin at the lower end of the dosing ranges used in rat studies though mice demonstrated this at higher doses and also showed evidence at the upper range of adrenal hypertrophy as well. In a rat study which contained both 4-week and 13-week recovery groups after 4-weeks of TAK-375 treatment this increase in adrenal weight and the adrenocortical vacuolization was still noted at 4-weeks after TAK-375 cessation but neither increased adrenal weight or vacuolization were present with a 13-week treatment-free period suggesting this finding is reversible. Adrenocortical vacuolization has been previously demonstrated to occur with inhibition of ACTH release, disrupted steroidogenesis and impairment in release of glucocorticoids from the adrenal gland (Rosol et

al., 2001; Capen, 2001). Melatonin itself has been shown to inhibit corticosterone release into circulation as well as reduce the pituitary response to corticotrophin releasing factor resulting in a decline in ACTH levels in circulation (Preslock, 1984). It is believed likely by the Sponsor that TAK-375 is causing this response in the adrenal gland by a similar direct pharmacologic action exerted through melatonin receptors in the pars tuberalis and pituitary as well as in the adrenal gland. The Sponsor did perform a dedicated study to examine the effects of TAK-375 administration for 1-week on the levels of circulating hormones from the pituitary and endocrine organs. Results indicated a trend towards reduction in ACTH levels when compared with controls though this change was not statistically significant. This reviewer considers the mechanism described by the Sponsor to be possible as the repeat-dose toxicology studies in which the adrenal gland findings were noted were of greater duration.

The adrenal gland of cynomolgus monkeys were not noted to exhibit overt or occult pathology with studies of greater than 4-weeks duration. Adrenal gland weight and structure were unaffected by treatment with TAK-375 for 4-weeks or 39-weeks though an increase in liver weight and adrenocortical hypertrophy characterized by decreased lipid in zona fasciculate cells was noted along with atrophy of the zona glomerulosa cells in monkeys used for dose range-finding studies of 2-weeks duration. It is not clear why these findings were not observed in the longer studies with oral TAK-375 administration at an identical dose level. The Sponsor does not provide an explanation as to this difference though physiologic or pharmacologic tolerance must be suspected. Studies with intravenous M-II did not detect an effect of this active metabolite on any parameter or organ including the adrenal gland in a 4-week study.

There is no reason to believe the findings in the adrenal gland are species specific; therefore, it appears this finding should be considered to be a potential risk with human exposure to TAK-375. However, NOAEL exposures in rodent are multiples of the exposure predicted in humans with therapeutic administration of Ramelteon. Also mitigating concern is the finding that the adrenocortical vacuolization in the rat demonstrates evidence of reversibility with cessation of TAK-375 administration.

Ovary

A similar demonstration of interstitial cell vacuolization was noted in the ovaries of rodents administered TAK-375. Rats appeared more sensitive to this effect when compared with mice. This too reversed in rats allowed to complete a 4-week or 13-week recovery period after a 4-week administration of TAK-375. A similar explanation as to the adrenal gland previously may be offered: melatonin has been shown to inhibit the release of gonadotropins from the pituitary (Preslock, 1984) and is the basis for seasonality in reproduction observed in species known to be seasonal breeders (Malpoux, 1999). Though effects on the ovary were not observed in the 4-week or 39-week monkey studies, this toxicity may not be species specific and could potentially be observed with human exposure in females though a significant exposure margin exists between human therapeutic levels and the exposures in rodents associated with the NOAEL.

Hematology and other clinical chemistry

The primary hematologic alteration associated with TAK-375 administration in all nonclinical species used in general toxicology studies was an elevation in platelet concentration. This is not believed to be a significant adverse finding as the changes were

relatively generally mild and evidence of altered thrombogenicity were not noted in short-term or chronic toxicity studies. The effect on platelets is believed related to the observation that melatonin may stimulate megakaryocytes and has been used experimentally as a treatment for IL-2 induced thrombocytopenia during cancer chemotherapy (Bregani et al., 1995; Lissoni et al., 1999). Microscopic examination of bone marrow in toxicology studies did not reveal an effect of TAK-375 on the cellular composition of this tissue. Mild anemia was noted in the 26-week rat study and demonstrated evidence of extramedullary hematopoiesis with microscopic examination of the spleen along with mild increases in reticulocytes. No evidence for anemia was observed in studies of the monkey. All other hematologic responses in nonclinical studies were mild and not consistent between studies or species and are believed by both Sponsor and this reviewer to be incidental and not reflective of a potential risk to humans.

In rats, the levels of serum Ca²⁺ were elevated in 4- and 13-week studies at the highest doses examined. Recovery was shown in the 4-week study with a 13-week treatment-free period. The Sponsor suggests this is likely a direct extension of the melatonin receptor agonist pharmacology of TAK-375 as melatonin has been demonstrated to decrease corticosterone release which reduces corticosterone-induced Ca²⁺ uptake into bone and therefore would promote higher circulating Ca²⁺ levels (Hakanson et al., 1990, Nishino et al., 1991; Hirsch et al., 1998). Although this would appear a plausible explanation, no significant change in circulating plasma corticosterone levels was demonstrated in a dedicated study of the plasma hormone levels in rats following 4-week oral administration of TAK-375.

The following table which was adapted from Sponsor details the generally wide margins between the NOAEL exposure in nonclinical studies and the expected therapeutic exposure to TAK-375 and metabolites with use of Ramelteon at the indicated 8 mg dose.

Exposure to TAK-375 and Metabolites at the Nonclinical NOAEL and the Margin of Exposure (MOE) over the Predicted Human Exposure* with Therapeutic Use of Ramelteon												
Species	Study Duration	NOAEL Dose (mg/kg/day)	TAK-375		M-II		M-I		M-III		M-IV	
			NOAEL AUC ₀₋₂₄ (ng·h/mL)	Human MOE	NOAEL AUC ₀₋₂₄ (ng·h/mL)	Human MOE	NOAEL AUC ₀₋₂₄ (ng·h/mL)	Human MOE	NOAEL AUC ₀₋₂₄ (ng·h/mL)	Human MOE	NOAEL AUC ₀₋₂₄ (ng·h/mL)	Human MOE
Mouse	13-Weeks	100 [†]	8681	924	7143	30	6665	833	28385	8253	9461	166
Rat	26-Weeks	10	1943	207	1336	6	1978	247	2914	833	1544	27
Rabbit	12-days [‡]	60	22000	2340	4769§	20	27858§	3482	4241§	1212	3375§	59
Monkey	39-Weeks	12	1626	173	12.6	0.05	1196	150	2378	679	14	0.25
	4-Weeks [‡]	30			8696	36						

* Estimated from study 01-01-TL-375-003 from exposure in elderly to 16 mg Ramelteon and assuming linear kinetics. AUC₀₋₂₄ for TAK-375, M-I, M-II, M-III and M-IV with a Ramelteon dose of 8 mg is estimated at 9.4, 8, 241, 3, 3.5, and 57 ng·h/mL. Note that in the table above the M-II metabolite is next to TAK-375 to keep the active parent and metabolite together for easier reference.

† The Sponsor's NOAEL in the study report M-11-00178.

‡ From the Segment II embryofetal development study in the rabbit.

§ From the 4-week repeat-dose toxicology study conducted with intravenous administration of M-II (25:85) to cynomolgus monkey.

¶ Estimated from toxicokinetic data in the rabbit at a dose of 300 mg/kg/day and assuming linear kinetics.

Coverage by the nonclinical NOAELs for the metabolite M-II is significantly lower than for TAK-375 and other metabolites with the exception of the metabolite M-IV in the monkey which is in fact below the expected human exposure. Although the lack of M-IV coverage in the monkey could be of some concern as this metabolite should be considered a major circulating metabolite in human and thus should have coverage from two species, including one non-rodent (and therefore is technically is only covered by rodents), the monkey exposure

to M-IV at the LOAEL dose in the 39-week study in which only clinical signs of vomiting, a decrease in APTT of questionable toxicologic significance, and a mild increase in liver weight without evidence of underlying histopathologic change were observed provides an exposure margin of 8-fold over the expected human exposure to this metabolite.

Reproductive Toxicology

Toxicity to male reproductive organs

A dedicated study of the effects of TAK-375 administration for 4-weeks on the plasma levels of testosterone and LH revealed a depressant effect on plasma levels of testosterone when assessed over 24 hr after the final dose while a weak effect on LH was observed sporadically during the day at 4.5 hr and 24 hr after the final administration. The toxicity of TAK-375 to male reproductive organs was studied as part the repeat-dose toxicology testing program in mice (4- and 13-week studies), rats (4-, 13-, and 26-week studies) and monkeys (4- and 39-week studies) and were completed without evidence of a deleterious effect on male reproductive organs. In addition, the M-II metabolite - which does not circulate to a great extent in monkeys - was given intravenously for 4-weeks and did not result in toxicity to reproductive organs. On balance TAK-375, despite its endocrine effects, does not appear to have direct toxicity to male reproductive organs.

Segment I: Fertility and early embryonic development to implantation

Evidence from fertility and early embryonic studies of the Sprague-Dawley rat indicates TAK-375 may suppress fertility through an effect on female reproductive function. Irregularity of the estrus cycle, decreased number of corpora lutea (high dose only), reduced number of implants, and reduced number of live embryos were noted and occurred in a dose-dependent manner. Administration of TAK-375 to males alone did not affect fertility parameters in a 2nd mating and no alterations in reproductive organ weights or structure when examined histologically were noted. Sperm motility, count and morphology were all normal which indicates the fertility impairment is related solely to effects on the female. A further repeat of the study with dosing in both male and female rats but using lower, but overlapping doses did not detect the reduction in implants or number of live embryos though the irregular estrus cycle was still evident. The NOAEL for fertility in these studies is considered to be 20 mg/kg/day for female and > 200 mg/kg/day for male rats. No toxicokinetic analysis was conducted to determine plasma exposure at 20 mg/kg/day dose in females. Conversion of this 20 mg/kg/day dose to body surface area provides for a 26-fold margin of exposure (based on a 65 kg individual) over the expected human therapeutic concentration of TAK-375 though it should be noted that the toxicokinetic assessment of TAK-375 in the plasma from a 6 mg/kg/day dose in the initial Segment I study would provide a multiple of 138-fold. It may therefore be expected that the 20 mg/kg/day dose level may provide an exposure margin much higher than determined by body surface area conversion though this number is not known. It must also be noted information on the plasma concentrations of M-II or other TAK-375 metabolites was absent from these study reports and thus the exposure margins based on a comparison of the active metabolite M-II in rat to the human exposure cannot be determined.

It is conceivable the toxicity to the ovary or other disruption of the hypothalamic-pituitary-gonadal axis and vacuolization of the ovaries found in rat studies may be the underlying dysfunction resulting in the decreased fertility parameters. Melatonin has been

reported to downregulate gonadotropin-releasing hormone gene expression in cell line by an action at the GnRH gene enhancer (Gillespie et al., 2004). A further explanation may involve the recent discovery of a hypothalamic neuropeptide named GnIH (gonadotropin-inhibiting hormone) located in the paraventricular nucleus with terminals in the median eminence which express melatonin receptors and evidence that melatonin may regulate quail reproduction through GnIH. The analogous neuropeptide has been cloned from hamster brain so this hormone may be more broadly found in mammals. Of note, however, is that a dedicated study of the effects of TAK-375 administration on prolactin and estradiol levels in female rats with dosing up to 250 mg/kg/day for 5 weeks did not reveal an effect on these hormones though a prolonged estrus cycle was noted with dosing ≥ 60 mg/kg/day, similar to that seen in the aforementioned studies.

Segment II: Embryofetal development studies

TAK-375 demonstrated evidence of teratogenicity in the Sprague-Dawley rat but this was not observed with TAK-375 exposure to the Japanese White rabbit in embryofetal development studies. In the rat, these abnormalities were both visceral and external and consisted of diaphragmatic hernia which began to be seen at the 150 mg/kg/day level, becoming statistically significant at 600 mg/kg/day at which dose level an additional finding of cysts on the external genitalia were noted. Variations observed to increase significantly in a dose-dependent manner included irregularly shaped scapula and ribs. Signs of clinical intoxication and other adverse effects in dams, notably transient decreases in weight and increased liver weight were noted at the same dose at which teratogenicity was observed in fetuses. The contribution of maternal toxicity to the structural abnormalities observed in the fetus is not known and therefore the presumption should be that a risk to the human fetus exists. Though M-II is found in the plasma of dams and exists at higher concentrations in the fetal plasma than in dams, this metabolite is unlikely to be the species involved in this effect as an intravenous embryofetal developmental study of M-II in the rat with administration up to 30 mg/kg/day failed to see similar malformations. The embryofetal and maternal NOAEL in the rat is identified at 40 mg/kg/day with AUC₀₋₂₄ exposures to TAK-375 and M-II approximately 1,892-fold and 45-fold the human exposure at expected therapeutic plasma levels. In the rabbit embryofetal development study, maternal toxicity included transient weight reduction and clinical signs with a maternal NOAEL of 60 mg/kg/day (AUC₀₋₂₄ exposure to TAK-375 and M-II approximately 2,340-fold and 45-fold the human, respectively). As no effects were observed in the fetuses, the embryofetal NOAEL in the rabbit is > 300 mg/kg/day with maternal AUC₀₋₂₄ exposure to TAK-375 and M-II approximately 7,602-fold and 99-fold, respectively, the human exposure. Taken together, evidence of embryofetal toxicity is apparent with teratogenicity in a single species though this occurs at a plasma concentration that provides a large safety margin over human exposure.

Segment III: Prenatal and postnatal development

Further effects of TAK-375 on reproduction were demonstrated by alterations in pre- and postnatal development in the Sprague-Dawley rat. Effects on F₁ litters were seen in F₁ offspring of litters at ≥ 100 mg/kg/day dose levels where maternal toxicity was evident. A dose-dependent reduction in body weight during and after weaning in litters of dose levels ≥ 100 mg/kg/day as well as evidence for delayed maturation in litters of the 300 mg/kg/day dose level which consisted of a reduced rate of differentiation for a measure of physical

development, eruption of lower incisors, and a reduced rate of acquisition of the righting reflex when compared with controls. Additional effects in the 300 mg/kg/day offspring appeared to be an alteration of emotionality as determined by an increased latency to movement in an open field test. Postmortem examination of F₁ offspring revealed an increased incidence of diaphragmatic hernia in the 300 mg/kg/day group litters which is consistent with the Segment II embryofetal development study described previously. No effect was observed on fertility and reproductive functions of F₁ offspring and no abnormalities were noted in the F₂ generation followed until weaning. The NOAEL for developmental toxicity in the rat was identified at 30 mg/kg/day. Toxicokinetics were not conducted in this study but by conversion of this dose to body surface area for comparison to human this would represent a 39-fold margin of exposure in a 65 kg individual.

The following table summarizes the margins of safety or exposure for human use of Ramelteon at 8 mg (based on the Sponsor's estimates of exposure from studies in the elderly) when compared to the nonclinical NOAEL dose or exposure in the reproductive toxicology studies.

Human Margins of Safety or Exposure over the Nonclinical NOAEL in Reproductive Toxicology Studies						
Study	Species	NOAEL Type	NOAEL Dose (mg/kg/day)	Margin of Safety or Margin of Exposure*		
				BSA [‡]	AUC _{0-24h}	
					TAK-375	M-II
Segment I	Rat	Fertility and early embryonic development	20	26-fold	n.d.	
Segment II	Rat	Maternal	40		1,892-fold	45-fold
		Embryofetal				
	Rabbit	Maternal	60	157-fold	2,340-fold†	n.d.
		Embryofetal	300		11,862-fold†	99-fold†
Segment III	Rat	F ₀ (maternal) F ₁ development	30	39-fold	n.d.	

*. Estimated from study 01-01-TL-375-003 from exposure in elderly to 16 mg Ramelteon and assuming linear kinetics. AUC₀₋₂₄ for TAK-375, M-I, M-II, M-III, and M-IV with a Ramelteon dose of 8 mg is estimated at 9.4, 8.2, 41.3, 3.5, and 57 ng·h/ml.

‡. Body surface area conversion based on a 65 kg individual

n.d., not determined

†. Based on toxicokinetic data from the original Segment II study (M-11-001003.001)

‡. Based on data from a follow-up toxicokinetic study in non-pregnant 13-week old female rats

Genetic Toxicology

A standard battery of genetic toxicity tests comprising a bacterial reverse mutation assay, *in vitro* chromosomal aberration assay in CHL cells, and *in vivo* micronucleus assays in mice and rats were conducted with TAK-375. Several additional tests including an *in vitro* cell gene mutation test and an *in vivo*, *in vitro* unscheduled DNA synthesis assay in rat hepatocytes were subsequently conducted for further informational support. The Sponsor also performed follow-up studies to demonstrate assay exposure to TAK-375 metabolites when the parent compound was incubated with the S9 liver fraction containing rat hepatic microsomes. All studies with the exception of the *in vitro* chromosomal aberration were judged to be negative for evidence of genotoxicity. The chromosomal aberration study conducted in CHL cells demonstrated an increase in structural and numerical aberrations with exposure to TAK-375 in direct assays of 24 and 48 hr as well as a +S9 activation condition. The lack of chromosomal

aberrations observed in the -S9 activation condition is of dubious validity as the positive control used, cyclophosphamide, requires metabolic activation to produce genotoxicity and thus failed in this condition. Overall, the weight of evidence might suggest TAK-375 is not genotoxic as the chromosomal aberration assay is known to generate false positives at a higher rate than other assays. Nevertheless, a genotoxic effect cannot be ruled out and should be included in the label as such unless a convincing explanation is brought forward by the Sponsor or the study is repeated without demonstration of clastogenicity and polyploidy.

Carcinogenicity

TAK-375 was administered by oral gavage to mice and rats for up to 104-weeks in order to evaluate the carcinogenic potential of this compound. The studies were considered adequate and valid by this reviewer and conclusions received concurrence from the Executive Carcinogenicity Assessment Committee.

Evidence of hepatic tumors characterized as hepatic adenoma and carcinoma were found in both male and female mice while male mice had an additional finding of hepatoblastoma at the highest dose used. The high dose, however, exceeded the MTD and required the early termination of this dose level in Week 95 with early mortality believed due to the hepatic tumor burden. Male mice appeared more sensitive to the development of hepatic tumors with TAK-375 administration which may be related to the higher TAK-375 and M-II plasma levels noted when compared with females. An apparent, though statistically non-significant, increase in Harderian gland adenoma was also observed in males and females at all dose levels when compared with control. The incidence did not increase over a 30-fold dose range though all values in the TAK-375-treated groups were above the incidence found in historical control data. Interpretation of this lack of dose-dependence in Harderian gland tumors is confounded by the early termination of the high dose groups, however, potentially obscuring a true effect of dose escalation. In general, however, tumors of the Harderian gland in the rodent are not considered to confer risk to humans as there is no analogous structure.

When considering the margin of safety for an assessment of human risk the hepatic tumor NOAEL exposure in the female mouse to TAK-375, the active and major metabolite M-II, and the inactive but major metabolite M-IV is over 800-fold higher, 12-fold and 104-fold above the expected human therapeutic plasma concentrations. For the male mouse, when hepatic adenoma and carcinoma are combined these safety margins are reduced to 103-fold, 3-fold, and 64-fold. Therefore, although the margin of safety for the parent is quite wide, the exposure difference between male and female mice at the NOAEL level is significantly lower for the M-II metabolite which is the major circulating species in the human.

Administration of TAK-375 to rats by oral gavage for up to 104-weeks was associated with the development of hepatic tumors consistent with the studies conducted in mice and characterized as hepatic adenoma and carcinoma in both males and females though in the rat the females appeared more sensitive to the development of hepatic adenoma. Perhaps explaining the difference in gender sensitivity the female rat was noted to have higher exposure to TAK-375 and the active metabolite M-II than males. In addition to the hepatic tumors, the male rat was noted to develop Leydig cell tumors of the testes at the high dose. As in the mouse study, the high dose in the rat bioassay resulted in excessive mortality and required the early termination of this group during Week 94. Premature mortality in the high dose developing later in the study was considered due to the hepatic tumor burden. The rat liver

tumor NOAEL corresponded to exposure levels of TAK-375, M-II, and M-IV that were 1,419-, 12-, and 27-fold higher than that expected in humans with the therapeutic dose of Ramelteon. The rat Leydig cell tumor NOAEL corresponded to exposure levels of TAK-375, M-II, and M-IV which were 4,472-, 14-, and 108-fold higher than the expected human exposure. As in the mouse studies, the margin of safety appears quite wide for TAK-375 exposure in the rat though this drops significantly when considering the margins of exposure for M-II between the rat and human.

The following tables delineate the margins of exposure between the NOAELs in the mouse and rat carcinogenicity bioassay with the expected human therapeutic exposure to TAK-375 and the active metabolite M-II.

Comparison of TAK-375 and M-II Exposures at the Mouse Tumor NOAEL versus the Expected Therapeutic Exposure with Ramelteon 8 mg/day in Humans						
Tumor/Type	SEX	NOAEL DOSE (mg/kg/day)	[TAK-375] (ng•h/mL)	HUMAN MOE†	[M-II] (ng•h/mL)	HUMAN MOE†
LIVER						
Adenoma	M	100	10,138	1,079-fold	7,203	30-fold
	F	100	7,773	827-fold	2,789	12-fold
Carcinoma	M	100	10,138	1,079-fold	7,203	30-fold
	F	300	21,061	2,241-fold	7,996	33-fold
Adenoma + Carcinoma	M	30	967	103-fold	614	3-fold
	F	300	7,773	827-fold	2,789	12-fold
Hepatoblastoma	M	100	10,138	1,079-fold	7203	30-fold
	F	>1000	48,829	5,195-fold	14,496	> 60-fold

†, MOE = Margin of Exposure, based on results from study 01-01-TL-375-03; elderly administered 16 mg with an extrapolation to 8 mg based on linear kinetics within this dose range. Estimated AUC_{0-24h} TAK-375: 9.4 ng•h/mL; M-II: 241.3 ng•h/mL.

Comparison of TAK-375 and M-II Exposures at the Rat Tumor NOAEL versus the Expected Therapeutic Exposure with Ramelteon 8 mg/day in Humans						
Tumor/Type	SEX	NOAEL DOSE (mg/kg/day)	[TAK-375] (ng•h/mL)	HUMAN MOE†	[M-II] (ng•h/mL)	HUMAN MOE†
LIVER						
Adenoma	M	60	13,432	1,419-fold	2,953	12-fold
	F	15	4,434	472-fold	3,773	16-fold
Carcinoma	M	250	42,033	4,472-fold	3,359	14-fold
	F	250	91,839	9,770-fold	22,278	92-fold
TESTIS						
Leydig cell tumor	M	250	42,033	4,472-fold	3,359	14-fold
	F			N/A		

†, MOS = Margin of Exposure, based on results from study 01-01-TL-375-03; elderly administered 16 mg with an extrapolation to 8 mg based on linear kinetics within this dose range. Estimated AUC_{0-24h} TAK-375: 9.4 ng•h/mL; M-II: 241.3 ng•h/mL.

The consistent development of hepatic tumors in rodents in the 2-year carcinogenicity bioassays implies a cross-species mechanism which merits potentially greater concern. The Sponsor provided evidence from both repeat-dose toxicology studies in mice and rats of a significant induction of hepatic microsomal enzymes involved in drug metabolism. This was further confirmed in dedicated one-week repeated-dose oral administration study of TAK-375 to mice and rats in which a broad panel of substrates for specific cytochrome P450 enzymes was screened and indicated significant induction. Drug-induced induction of hepatic drug-metabolizing enzymes has been suggested and generally accepted as the mechanism behind phenobarbital-induced hepatocellular adenoma and carcinoma in mice and rats (Whysner et al., 1996). TAK-375 administration for 1-week to mice and rats produced a pattern and magnitude of enzyme induction which paralleled that of phenobarbital. According to the current opinion of the scientific community, the induction of hepatic microsomal enzymes as a mechanism for the development of hepatic tumors in rodents is not thought to be operative in humans and serves to mitigate this reviewer's concern. The Sponsor did offer an alternative explanation which involved a mechanism by which TAK-375 may increase dehydroepiandrosterone (DHEA) levels as melatonin has been demonstrated to stimulate DHEA levels both *in vitro* and *in vivo*. DHEA has been reported to be a peroxisome proliferator therefore may lead to the development of tumors through this route though it has also been shown to induce hepatic microsomal enzymes. Notably however, no evidence of peroxisome proliferation was found from histopathologic reports. Further evidence this mechanism is unlikely to be involved was a dedicated 4-week repeat-dose study designed to determine the effect of TAK-375 on plasma melatonin and DHEA levels and to correlate this to ultrastructural changes in hepatocytes using dose levels identical to the rat study. TAK-375 administration produced an elevation in circulating melatonin and DHEA levels but on microscopic examination the changes in hepatocytes were limited to expansion of the smooth endoplasmic reticulum consistent with hepatic enzyme induction and no evidence of peroxisome proliferation was noted. Additionally, the activity of acyl-CoA oxidase, a marker for peroxisome proliferation, was not increased at any dose level. Taken as a whole, the evidence appears to support the hypothesis that the hepatic tumors observed in rodents are a consequence of significant induction of hepatic microsomal enzymes, a mechanism which is unlikely to lead to tumors in humans as the clinical experience with hepatic enzyme inducing drugs such as phenobarbital indicates an absence of risk.

The development of Leydig cell tumors in the rat was proposed by the Sponsor to be a result of inhibition of testosterone by TAK-375 and a consequent release from feedback inhibition on LH release from the pituitary resulting in overstimulation of the Leydig cells and development of hyperplasia and adenoma. This mechanism is considered plausible as compounds which interfere with testosterone synthesis or are GnRH agonists or promote release of this hormone are associated with an increase in LH levels and the development of this tumor. Notably, demonstration of this mechanism would not be considered to confer great risk to humans as the sensitivity of human Leydig cells to LH has been shown to be much weaker than the rat (Cook et al., 1999). The Sponsor provided evidence that daily oral administration of TAK-375 for 1-week or 4-weeks to rats produced a suppression of testosterone synthesis at dose levels at which were associated with Leydig cell hyperplasia and adenoma in the 2-year bioassay. Weaker evidence, however, was demonstrated for an elevation in circulating LH levels. Though an elevated LH was found in the 4-week study

using a large sample size, no evidence was found in the 1-week study and the time-points at which LH differed significantly from control animals in the 4-week study were only at 4.5 hr (and not at 4 hr or 5 hr) and at 24 hr after the final administration of TAK-375. Thus, the mechanism proposed by the Sponsor has the benefit of some support from their data but definitive evidence for this explanation is, in this reviewer's opinion, lacking. Nevertheless, the development of this tumor in the rat occurs with an exposure level which affords a large margin of safety for human use.

Though the epigenetic mechanisms proposed by Sponsor to underlie the development of hepatic tumors in rodents and Leydig cell tumors in rats are plausible and evidence of varying quality to support these mechanisms have been provided, a genotoxic mechanism is also possible. As discussed previously TAK-375, and potentially the metabolites as well, were demonstrated to produce a positive clastogenic response in an *in vitro* chromosomal aberration assay test under several experimental conditions. Though the remaining *in vitro* and *in vivo* genotoxicity assays did not indicate potential for a mutagenic or clastogenic effect, the potential involvement of a genotoxic mechanism in the development of rodent tumors cannot be entirely excluded.

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2.6.6.10 Tables and Figures

Table 4.a Toxicokinetic Data for Ramelteon in Mice, Rats, Rabbits, and Monkeys at the Nonclinical NOAEL

Species	Dose Duration	NOAEL (mg/kg/day)	NOAEL (mg/m ²)	AUC 0-24hr (ng·hr/mL)	Animal:Human Ratio(a)	Report No.
Mouse	13 weeks	30	90.48	1288	137	M-11-178 [115]
Rat	26 weeks	10	59.04	1943	207	M-11-176 [116]
Rabbit	GD 6-18	60	755.95	22000	2340	M-11-103-1 [121]
Monkey	39 weeks	12	146.67	1626	173	M-11-232 [90]

GD, gestation days.

(a) AUC: animal at NOAEL, human at clinical dose in an elderly subpopulation (estimated ramelteon AUC value 9.4 ng·hr/mL; assumed linear pharmacokinetics for estimation of the 8 mg (4.67 mg/m²) exposure value. [82,107].

Table 4.b Toxicokinetic Data for M-I in Mice, Rats, Rabbits, and Monkeys at the Nonclinical NOAEL

Species	Dose Duration	NOAEL (mg/kg/day)	NOAEL (mg/m ²)	AUC (0-24hr) (ng·hr/mL)	Animal:Human Ratio(a)	Report No.
Mouse	13 weeks	30	90.48	2117	265	M-11-178 [115]
Rat	26 weeks	10	59.04	1978	247	M-11-176 [116]
Rabbit	GD 6-18	60	755.95	27858(b)	3482	M-11-188 [95]
Monkey	39 weeks	12	146.67	1196	150	M-11-232 [90]

GD, gestation days.

(a) AUC: animal at NOAEL, human at clinical dose in an elderly subpopulation estimated M-I AUC value 8.0 ng·hr/mL; assumed linear pharmacokinetics for 8 mg (4.67 mg/m²) exposure value. [82,107].

(b) Estimated from M-I AUC value from rabbits administered 300 mg/kg/day; assumed linear pharmacokinetics over dose range.

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Table 4.c Toxicokinetic Data for M-II in Mice, Rats, Rabbits, and Monkeys at the Nonclinical NOAEL

Species	Dose Duration	NOAEL (mg/kg/day)	NOAEL (mg/m ²)	AUC (0-24hr) (ng-hr/mL)	Animal:Human Ratio(a)	Report No.
Mouse	13 weeks	30	90.48	875	3.6	M-11-178 [115]
Rat	26 weeks	10	59.04	1336	5.5	M-11-176 [116]
Rabbit	GD 6-18	60	755.95	4769(b)	19.8	M-11-188 [95]
Monkey	39 weeks	12	146.67	12.6	0.05	M-11-232 [90]
	4 weeks (c)	10 (d)	122.22	8696	36	M-11-00192.001 [192]

GD, gestation days.

(a) AUC: animal at NOAEL, human at clinical dose in an elderly subpopulation (estimated M-II AUC value 241.3 ng-hr/mL; assumed linear pharmacokinetics for 8 mg (4.67 mg/m²) exposure value, [82,107].

(b) Estimated from M-II AUC value from rabbits administered 300 mg/kg/day; assumed linear pharmacokinetics over dose range.

(c) Based on results of a 4-week intravenous toxicity study in monkeys with M-II (2S,8S) [192].

(d) M-II (2S,8S) NOAEL.

Table 4.d Toxicokinetic Data for M-III in Mice, Rats, Rabbits, and Monkeys at the Nonclinical NOAEL

Species	Dose Duration	NOAEL (mg/kg/day)	NOAEL (mg/m ²)	AUC (0-24hr) (ng-hr/mL)	Animal:Human Ratio(a)	Report No.
Mouse	13 weeks	30	90.48	6310	1802	M-11-178 [115]
Rat	26 weeks	10	59.04	2914	833	M-11-176 [116]
Rabbit	GD 6-18	60	755.95	4241(b)	1212	M-11-188 [95]
Monkey	39 weeks	12	146.67	2378	679	M-11-232 [90]

GD, gestation days.

(a) AUC: animal at NOAEL, human at clinical dose in an elderly subpopulation (estimated M-III AUC value 3.5 ng-hr/mL; assumed linear pharmacokinetics for 8 mg (4.67 mg/m²) exposure value, [82,107].

(b) Estimated from M-III AUC value from rabbits administered 300 mg/kg/day; assumed linear pharmacokinetics over dose range.

Table 4.e Toxicokinetic Data for M-IV in Mice, Rats, Rabbits, and Monkeys at the Nonclinical NOAEL

Species	Dose Duration	NOAEL (mg/kg/day)	NOAEL (mg/m ²)	AUC (0-24hr) (ng-hr/mL)	Animal:Human Ratio(a)	Report No.
Mouse	13 weeks	30	90.48	2597	45.6	M-11-178 [115]
Rat	26 weeks	10	59.04	1544	27.1	M-11-176 [116]
Rabbit	GD 6-18	60	755.95	3375(b)	59.2	M-11-188 [95]
Monkey	39 weeks	12	146.67	14.0	0.25	M-11-232 [90]

GD, gestation days.

(a) AUC: animal at NOAEL, human at clinical dose in an elderly subpopulation (estimated M-IV AUC value 57 ng-hr/mL; assumed linear pharmacokinetics for 8 mg (4.67 mg/m²) exposure value, [82,107].

(b) Estimated from M-IV AUC value from rabbits administered 300 mg/kg/day; assumed linear pharmacokinetics over dose range.

Table 5. a AUC Exposure Margins Table at Nonclinical NOAELs Relative to Human Exposures(a)

	Mouse	Rat	Rabbit (b)	Monkey
Ramelteon	137	207	2340	173
M-I	265	247	3482	150
M-II	3.6	5.5	19.8	0.05 (c) 36 (d)
M-III	1802	833	1212	679
M-IV	45.6	27.1	59.2	0.25

(a) At the proposed clinical dosage of 8 mg, estimated human AUC values for ramelteon, M-I, M-II, M-III and M-IV are 9.4, 8.0, 241.3, 3.5, and 57 ng-h/mL, respectively.

(b) Metabolite values estimated from rabbits administered 300 mg/kg/day.

(c) Exposure multiple was calculated based on the results of a 39 week oral toxicity study of ramelteon in monkeys; NOAEL of 12 mg/kg/day.

(d) Exposure multiple was calculated based on the results of a 4-week intravenous toxicity study in monkeys with M-II (2S,8S); NOAEL of 10 mg/kg/day.

2.6.7 TOXICOLOGY TABULATED SUMMARY

See Appendix 2 Sponsor's tabulation of all major toxicology studies.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: TAK-375 has been adequately characterized in nonclinical pharmacokinetic and toxicologic studies including repeat-dose toxicology, genetic toxicology, carcinogenicity, and reproductive toxicology studies such that the risks for human use may be adequately judged. The No Adverse Effect Levels (NOAELs) in the pivotal toxicology studies provide a wide margin of exposure or safety for human use at the indicated dose based on the parent compound, TAK-375 and a moderate margin for the active metabolite M-II. The metabolism of TAK-375 in nonclinical species used for toxicology studies were qualitatively similar to human with the exception of the monkey which produces little M-II or the inactive metabolite M-IV with oral administration of TAK-375. M-II was evaluated in the monkey with intravenous administration of the isolated metabolite for up to 4 weeks which is considered acceptable and no toxicity was exhibited at a dose which provides coverage for human exposure. The inactive metabolite M-IV based on current draft guidelines is considered a major human metabolite and was adequately covered by a chronic rodent study though not by a non-rodent study. The Sponsor was not informed of this draft policy during development and the non-rodent chronic LOAEL demonstrated mild adverse effects without histopathologic findings and provides a margin of exposure of 8-fold. M-IV was evaluated within the genetic and reproductive toxicology studies as well as the 2-year carcinogenicity bioassays in mice and rat. This reviewer therefore believes no further characterization of the M-IV metabolite is required for approval.

The toxicities associated with oral administration of TAK-375 primarily involved signs of CNS sedation consistent with an exaggerated pharmacologic effect, increased liver weight with evidence of centrilobular hypertrophy in rodents and increased hepatic microsomal enzyme activities, increased thyroid weight with evidence of follicular cell hypertrophy in

rodents, adrenocortical and ovarian interstitial cell vacuolization in rodents, elevated platelets, and in rodents a mild increase in Ca^{2+} . The Sponsor provided evidence that TAK-375 and/or metabolites were potent hepatic enzyme inducers on par with phenobarbital and microscopic examination of rodent liver tissue demonstrated an increase in smooth endoplasmic reticulum consistent with this explanation. Thus, the increased liver weight and hepatocellular hypertrophy is considered adaptive and not adverse in nature in the rodent. Increased liver weight in the cynomolgus monkey was not considered due to hepatic enzyme induction but was adverse as indicated by a significant rise in ALT levels and non-zonal hypertrophy with chronic administration at a high dose. Thyroid weight increase and follicular hypertrophy in rodents was suggested by the Sponsor to be secondary to the increased metabolic activity of the liver, reduction of circulating thyroid hormones, and consequent increased release of TSH causing overstimulation of the rat thyroid resulting in hypertrophic response. Demonstration of the key hormone perturbations described was shown in rodent toxicology studies so this appears to be a plausible explanation. Explanation of the adrenal and ovarian effects as being due to an expected pharmacologic inhibition of the pituitary release of ACTH or gonadotropins, respectively, or disruption of synthesis and release of steroid hormones from these end organs is conceivable based on the known pharmacologic effects of melatonin but the data provided by the Sponsor provide rather limited support for this argument. Nevertheless, these effects have been shown to recovery fully in 4-week repeat dose toxicology studies in rats and these effects were not observed in the monkey. Mild elevation in platelets in rodent and monkey toxicology studies as well as increased Ca^{2+} levels in rodents were also explained as part of the published knowledge of the pharmacologic effect of melatonin and therefore to be expected. Mechanistic data were not provided to support this contention though recovery from these perturbations with cessation of dosing was demonstrated in the rat.

TAK-375 and/or its metabolites were studied in *in vitro* and *in vivo* genetic toxicology assays and were negative in all but the *in vitro* chromosomal aberration assay in Chinese hamster lung cells. A positive clastogenic response was observed both with and without metabolic activation and was characterized by both numerical and structural abnormalities. The study was considered of questionable validity on review due to the inappropriate use of a control which confounded the interpretation of one condition of the assay. This is not considered an approvability issue though the Sponsor may wish to repeat this assay to correct the methodologic problems evident.

Two-year carcinogenicity bioassays in the mouse and rat revealed hepatic tumors in both species which the Sponsor attributed to the ability of TAK-375 to induce hepatic microsomal enzymes, a mechanism which has previously been linked to the development of hepatic tumors in both mouse and rat. Though this mechanism is considered the likely causative factor and is not considered relevant to human risk, the finding of a clastogenic response in a genetic toxicity assay does not preclude a genotoxic mechanism for tumor development. If this were true, a direct genotoxicant would be expected to act across species and would be considered evidence of risk for human use. An additional finding of Leydig cell tumors in the rat was considered secondary to a reduction in circulating testosterone causing elevated LH release and overstimulation of these cells. Although dedicated plasma hormone studies were conducted and revealed a reduced testosterone level with oral administration of TAK-375 in rats, the evidence for increased LH was present but weak. Though an epigenetic mechanism appears the most logical and would not be expected to confer great risk for human

use due to lesser sensitivity to the proliferative effects of LH in humans, as described previously a genotoxic mechanism cannot be excluded.

Reproductive toxicology studies revealed an effect of TAK-375 on the estrus cycle which did not affect fertility though reduced numbers of implants and live embryos were noted with high dose levels in the rat. TAK-375 was teratogenic in the rat causing diaphragmatic hernia and cysts on the external genitalia. Increased incidence of variations primarily involved alterations in the skeleton including rib, sternbra, and vertebral variations. TAK-375 did not produce a notable effect in embryofetal development studies in the rabbit, however. A pre- and post-natal development study in the rat indicated reductions in F₁ body weight, delays in physical, neurological and alteration in emotional responses as well as confirming a propensity to produce diaphragmatic hernia. The reproductive capacity of the F₁ generation was not affected and the resulting F₂ generation was judged normal on all parameters.

Unresolved toxicology issues: All toxicities noted in the nonclinical program were observed at plasma concentrations of TAK-375 and its active M-II metabolite that exceeded by several to several thousand multiples the expected human exposure to these compounds. Other issues which remain to be clarified or addressed include the following:

Genetic toxicity

The positive finding in the *in vitro* chromosomal aberration assay suggests the possibility TAK-375 and/or metabolites are genotoxic though methodologic problems were revealed in the performance of this assay. Repeat of this assay using CHL cells or another system may resolve the issue of genetic toxicity and, if found negative on repeat, would suggest the tumors observed in the 2-year bioassays in mice and rats are due to epigenetic mechanisms which would be less likely to confer risk with human use.

The positive finding in the *in vitro* study is real; however, the weight of evidence indicates a low level of concern for *in vivo* genotoxicity. Therefore, this issue is not considered an approvability issue, as long as the sponsor agrees to include the positive finding in the label. Should the sponsor wish to conduct mechanistic studies to clarify the positive finding, labeling may then be reconsidered.

Metabolites

The M-IV metabolite which was not fully covered at the NOAEL dose in the chronic toxicity study in monkey should be evaluated by performance of safety pharmacology studies with the isolated metabolite as well as administration of the isolated metabolite to this species to complete qualification of this metabolite. In addition, the active M-II metabolite was not fully characterized in *in vitro* or *in vivo* cardiovascular safety pharmacology studies and should be evaluated.

As described in the text of the review, this is not considered to be an approvability issue; however, the sponsor is encouraged to characterize this metabolite in accordance with the draft guidance document: "Guidance for Industry – Safety Testing of Drug Metabolites."

Recommendations: From the pharmacology/toxicology perspective, this NDA may be approved.

Suggested labeling: See executive summary.

Signatures (optional):

Reviewer Signature Adam Wasserman, Ph.D.

Supervisor Signature R. Daniel Mellon, Ph.D. Concurrence Yes X No

APPENDIX/ATTACHMENTS

Appendix 1: Minutes of the Executive Carcinogenicity Assessment Committee on May 9, 2000.

Appendix 2: Review of Amendment #024 by Aisar Atrakchi, Ph.D. dated February 13, 2002

Appendix 3: Minutes of the Executive Carcinogenicity Assessment Committee on March 15, 2005.

Appendix 4: Tabulated summary of toxicology studies as extracted from the Sponsor's submission.

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PLEASE SEE APPENDICES 1 AND 2
INCORPORATED IN THE REVIEW(S)
DISCUSSING THE CARCINOGENICITY
STUDIES

APPENDIX 3

Executive CAC

Date of Meeting: March 15, 2005

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Lois Freed, Ph.D., HFD-120, Alternate Member
Daniel Mellon, Ph.D., HFD-170, Team Leader
Adam Wasserman, Ph.D., HFD-170, Presenting Reviewer

Author of Draft: Adam Wasserman

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 #: 21-782
Drug Name: TAK-375 (Ramelteon)
Sponsor: Takeda Global Research & Development Center, Inc.

Background:

TAK-375 acts as an agonist at melatonin (ML-1) receptors and is being developed by Takeda for the treatment of insomnia. TAK-375 is rapidly absorbed but has low oral bioavailability (approximately 6% in rats, 2% in humans) thought to be due to extensive first pass metabolism. TAK-375 has four significant Phase I metabolites in humans. One metabolite, M-II, is pharmacologically active and appears to have 10-100 fold lower potency at ML-1 receptors. In humans, the M-II metabolite circulates at approximately 20 to 40-times higher concentration than the parent and thus may contribute significantly to drug efficacy. In the rat, the M-II metabolite circulates at 10-20% of the parent concentration while in the mouse the M-II metabolite circulates at approximately 30% of the parent value. Plasma concentrations of the M-II metabolite achieved in both mice and rats in the 2-year bioassays exceeded the expected human therapeutic exposure to the metabolite. Completion of the genetic toxicology battery indicated the potential of the TAK-375 to be genotoxic in one assay. Incubation of CHL fibroblasts with TAK-375 produced evidence of chromosomal abnormalities including aneuploidy at the highest concentration tested; a concentration at which a precipitate was formed. TAK-375 was not found to be mutagenic in a bacterial reverse mutation (Ames) assay or a gene mutation assay using mouse LY1578 TK⁺ lymphoma cells. TAK-375 exhibited no evidence of clastogenicity in *in vivo* mammalian micronucleus assays in rats and mice. A subsequent study of TAK-375 in an *in vitro* UDS assay in rats/rat hepatocytes was negative; no increase in net nuclear grains was observed in treated animals when compared with controls. The Sponsor submitted the dosing strategy for the mouse and rat carcinogenicity bioassays based on an estimate of the MTD from several dose range-finding and repeat-dose toxicology studies. Notably, concurrence was

requested after the initiation of these studies. The Executive CAC was unable to concur with the doses selected for the mouse study due to concerns that the highest doses selected would be above the MTD. Regarding the rat study, the Executive CAC was concerned that the MTD was not clearly identified in an appropriate range-finding study or repeat-dose study of sufficient duration.

Mouse Carcinogenicity Study: TAK-375 was administered by oral gavage to B6C3F1 mice at doses of 0 (0.5% methylcellulose vehicle), 30, 100, 300 or 1000 mg/kg/day for up to 104 weeks. Significantly lower body weight relative to controls and high mortality in both males and females at the high dose led the Sponsor to terminate these groups during the 95th week of the study. Exposure to TAK-375 was associated with dose-dependent development of hepatic tumors including adenoma, carcinoma and hepatoblastoma in both males and females with the incidence of hepatic tumors being statistically different from controls at doses of ≥ 100 mg/kg/day in males and at doses of ≥ 300 mg/kg/day in females.

Rat Carcinogenicity Study: TAK-375 was administered by oral gavage to Sprague-Dawley rats at doses of 0 (0.5% methylcellulose vehicle), 15, 60, 250 and 1000 mg/kg/day for up to 104 weeks. Significantly lower body weight relative to controls occurred in both male and female rats administered the high dose and significantly greater mortality was observed in females. The high mortality led the Sponsor to terminate the high dose females in the 94th week of the study; the male high dose rats continued to be given the test article until study completion. Exposure to TAK-375 was associated with the dose-dependent development of hepatic tumors including adenoma and carcinoma in both males and females and Leydig cell tumor in males. Hepatic tumors were significantly increased above control animals at doses of ≥ 250 mg/kg/day in males and ≥ 60 mg/kg/day in females while Leydig cell tumors were significantly increased in the 1000 mg/kg/day group only.

Executive CAC Recommendations and Conclusions:

Mice:

1. The Committee accepted the carcinogenicity study as adequate.
2. The Committee concluded that there were dose-dependent hepatic tumors in both males (hepatic adenoma, carcinoma and hepatoblastoma) and females (hepatic adenoma and carcinoma). The Committee concluded that the relevance of these tumors to humans cannot be excluded.

Rats:

1. The Committee accepted the carcinogenicity study as adequate.
2. The Committee concluded that there was evidence of drug-induced neoplasms in both male and female rats. Both sexes demonstrated dose-dependent hepatic adenomas and carcinomas, and males also had Leydig cell tumors. The relevance of these tumors to humans cannot be excluded.

The Committee noted that the tumor findings in mice and rats may be a result of epigenetic factors; however, the possibility of a genotoxic mechanism cannot be completely excluded because of the findings in the in vitro chromosomal aberration assay. The Committee noted that more information on this issue might be gleaned from further analysis of already collected tissues.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\n
Division File, HFD-170
R. Daniel Mellon, Ph.D., Team leader, HFD-170
Adam Wasserman, Ph.D., Reviewer, HFD-170
Sara Stradley, CSO/PM, HFD-170
Adele Seifried, HFD-024

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this page is the manifestation of the electronic signature.**

/s/

David Jacobson-Kram
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APPENDIX 4

Ramelteon

2.6.7.7A Repeat Dose Toxicity: Four-week Oral Gavage Toxicity Study of TAK-375 in Rats

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2.6.7.7A Repeat-Dose Toxicity: Four-week Oral Gavage Toxicity Study of TAK-375 in Rats

Species/Strain:	— CD(SD)IGS Rats		Duration of Dosing:	4 Weeks		Test Article	TAK-375			
Initial Age:	6 Weeks		Duration of Postdose:	None		Study/Report No.:	2699/SU/M-11-27			
Date of First Dose:	24 Mar 98		Method of Admin.:	Gavage		GLP Compliance:	Yes			
Vehicle/Formulation:	0.5 w/v% methylcellulose solution									
Special Features:	Plasma T3, T4 and TSH levels Activity of hepatic drug-metabolizing enzymes (aniline hydroxylase and aminopyrine-N-demethylase) Activity of hepatic enzymes: UDP-glucuronosyltransferase and sulfotransferase									
No Observed Adverse Effect Level:	10 mg/kg/day									
Daily Dose (mg/kg)	0 (Control)		10		40		150		600	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Toxicokinetics (N: 4)										
Cmax (µg/mL)	NA	NA	2.66	1.27	2.55	7.03	4.19	6.08	6.11	37.71
AUC (µg·hr/ml)	NA	NA	1.4	1.0	4.0	10.2	23.2	33.4	72.0	204.5
Noteworthy Findings										
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0	0	0
Body Weight (%) Day 28 (a)	351 g	-	0	-	+3.4	-	-2.3	-	-7.1*	-
Food Consumption	-	-	-	-	-	-	-	-	-	↓
Water Consumption (N: 5)	-	-	-	-	-	-	-	-	-	-
Clinical Observations										
Salivation	0	0	0	0	0	0	3	3	9	9
Chromaturia	0	0	0	0	0	0	0	0	10	8
Ophthalmoscopy (N: 5)	-	-	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-	-	-
Clinical Chemistry										
Total cholesterol (mg/dL)	-	74	-	83	-	104*	-	106**	-	167**
Total protein (g/dL)	6.09	6.24	5.89	6.34	5.95	6.32	6.11	6.37	6.66**	6.76**
Calcium (mg/dL)	10.31	10.05	10.17	10.13	10.34	10.11	10.34	10.21	10.59*	10.68**
Triglyceride (mg/dL)	-	37	-	41	-	38	-	43	-	92**

2.6.7.7A Repeat-Dose Toxicity: Four-week Oral Gavage Toxicity Study of TAK-375 in Rats (continued)

Daily Dose (mg/kg)	0 (Control)		10		40		150		600	
	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Number of Animals										
Urinalysis (N: 5)	-	-	-	-	-	-	-	-	-	-
Organ Weights (%) (a)										
Thyroid gland (mg)	26.0	-	+8.8	-	+22.3**	-	+24.6**	-	+11.2	-
Adrenal gland (mg)	-	55.7	-	+6.1	-	+5.6	-	+19.0*	-	+40.0**
Liver (g)	9.45	5.74	-3.5	-1.0	+4.2	-1.9	+8.0	+14.6	+38.6**	+71.1**
Gross Pathology										
Dark discolored liver	-	0	-	0	-	0	-	2	-	10
Enlarged liver	0	0	0	0	0	0	0	0	9	4
Enlarged adrenal gland	0	0	0	0	0	0	0	2	3	7
Histopathology										
Adrenal gland:										
Cortical cell vacuolization	0	0	0	0	2	0	4	0	9	8
Minimal	0	0	0	0	2	0	4	0	6	6
Mild	0	0	0	0	0	0	0	0	3	2
Liver:										
Hepatocellular hypertrophy	0	0	0	0	0	0	5	5	10	10
Minimal	0	0	0	0	0	0	5	5	5	2
Mild	0	0	0	0	0	0	0	0	5	8
Ovary:										
Interstitial cell vacuolization	NA	0	NA	0	NA	0	NA	2	NA	5
Minimal		0		0		0		2		5

APPENDIX 4 (cont.)

Ramelteon

2.6.7.7A Repeat Dose Toxicity: Four-week Oral Gavage Toxicity Study of TAK-375 in Rats

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2.6.7.7A Repeat-Dose Toxicity: Four-week Oral Gavage Toxicity Study of TAK-375 in Rats (continued)

Daily Dose (mg/kg)	0 (Control)		10		40		150		600	
	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Additional Examinations										
T3 (ng/mL)	-	1.49	-	1.28	-	1.36	-	1.19**	-	1.12**
T4 (µg/dL)	4.35	-	4.50	-	4.60	-	4.77	-	5.15*	-
TSH (ng/mL)	3.39	2.07	2.62	1.29*	2.55	2.08	3.67	2.16	2.11**	4.11**
Aniline hydroxylase; (N: 5) (mU/mg protein)	0.108	0.104	0.131	0.095	0.104	0.103	0.144*	0.138	0.148*	0.177**
Aminopyrine-N-demethylase; (N: 5) (mU/mg protein)	1.535	0.900	1.546	0.834	1.354	0.877	1.572	1.071	1.926*	1.652**
Hepatic UGT for T4; (N: 5) (pmol/mg protein/min) (b)	1.63	1.65	NE	NE	NE	NE	NE	NE	2.27 ^{SS}	3.00 ^{SS}
Hepatic UGT for 4-nitrophenol; (N: 5) (nmol/mg protein/min) (b)	16.4	8.34	NE	NE	NE	NE	NE	NE	50.94 ^{SS}	45.14 ^{SS}

- indicates no noteworthy findings; Dunnett's Test: *, $p \leq 0.05$; **, $p \leq 0.01$; Student t-Test: \$\$, $p \leq 0.01$; ↓, tended to be decreased; NA, not applicable; NE, Not examined; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone; UGT, UDP-glucuronosyltransferase; ST, Sulfotransferase.

(a) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not the percent differences).

(b) Non-GLP.

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2.6.7.7B Repeat-Dose Toxicity:

Four-Week Oral Gavage Toxicity Study of TAK-375 in Rats with a 4- and 13-Week Recovery Period

2.6.7.7B Repeat-Dose Toxicity:

Four-Week Oral Gavage Toxicity Study of TAK-375 in Rats with a 4- and 13-Week Recovery Period

Species/Strain:	— :CD(SD)IGS Rats	Duration of Dosing:	4 Weeks	Test Article	TAK-375
Initial Age:	6 Weeks	Duration of Postdose:	4 and 13 Weeks	Study/Report No.:	2784/SU/M-11-110
Date of First Dose:	18 Aug 98	Method of Administration:	Gavage	GLP Compliance:	Yes
Special Features:	Plasma T3, T4, and TSH levels			Vehicle/Formulation:	0.5 w/v% methylcellulose solution
Conclusion:	All toxicologically significant changes were reversible				
Daily Dose (mg/kg)	0		600		
Number of Animals	M: 30	F: 30	M: 30	F: 30	
Noteworthy Findings					
Died or Sacrificed Moribund	0	0	0	0	
Body Weight (%) (a)	365	-	-4.4**	-	
Food Consumption (%)	-	-	-	↓	
Clinical Observations					
Salivation	0	0	26	22	
Chromaturia	0	0	30	27	
End of treatment					
Number Evaluated	10	10	10	10	
Hematology	-	-	-	-	
Clinical Chemistry					
Triglycerides (mg/dL)	43	35	74**	86**	
Cholesterol (mg/dL)	70	73	112**	164**	
Total protein (g/dL)	5.98	6.19	6.42**	6.64**	
Organ Weight (%) (a)					
Adrenal gland (mg)	52.0	65.0	+18.1*	+13.7*	
Liver (g)	9.48	5.83	+48.3**	+68.8**	

APPENDIX 4 (cont.)

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2.6.7.7B Repeat-Dose Toxicity:

Four-Week Oral Gavage Toxicity Study of TAK-375 in Rats with a 4- and 13-Week Recovery Period

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2.6.7.7B Repeat-Dose Toxicity:

Four-Week Oral Gavage Toxicity Study of TAK-375 in Rats with a 4- and 13-Week Recovery Period (continued)

Daily Dose (mg/kg)	0		600	
	M: 30	F: 30	M: 30	F: 30
End of treatment (continued)				
Number Evaluated	10	10	10	10
Gross Pathology				
Adrenal gland enlarged	0	0	3	4
Liver enlarged	0	0	10	10
Dark discoloration of liver	0	0	8	9
Histopathology				
Adrenal Gland				
Cortical cell vacuolization	0	0	10	9
Minimal	0	0	4	9
Mild	0	0	4	0
Moderate	0	0	2	0
Liver				
Hepatocellular hypertrophy	0	0	10	10
Minimal	0	0	8	7
Mild	0	0	2	3
Intracytoplasmic inclusion bodies	0	0	0	1
Ovary				
Vacuolization of interstitial cells	NA	0	NA	6
Minimal		0		6
Additional Examinations:				
T3 (ng/mL)	1.12	1.51	0.98**	0.94**
TSH (ng/mL)	-	0.84	-	2.36**

2.6.7.7B Repeat-Dose Toxicity:

Four-Week Oral Gavage Toxicity Study of TAK-375 in Rats with a 4- and 13-Week Recovery Period (continued)

Daily Dose (mg/kg)	0		600	
	M: 30	F: 30	M: 30	F: 30
Number of Animals				
Postdose Evaluation (4 Weeks)				
Number Evaluated	20	20	20	20
Clinical Observations				
Chromaturia (Week 1)	0	0	17	16
Clinical Chemistry (N: 10)				
Total protein (g/dL)	-	6.43	-	6.80*
Organ Weight (%) (N: 10) (a)				
Liver (g)	-	6.10	-	+13.8*
Histopathology (N: 10)				
Adrenal gland				
Cortical cell vacuolization	0	0	3	2
Minimal			3	2
Postdose Evaluation (13 Weeks)				
Number Evaluated	10	10	10	10
No noteworthy Observations	10	10	10	10

- indicates no noteworthy findings; Dunnett's Test: *, $p \leq 0.05$; **, $p \leq 0.01$; ↓, tended to be decreased; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone; NA, not applicable.

(a) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not the percent differences).

2.6.7.7C Repeat-Dose Toxicity: Twenty-Six-Week Oral Gavage Toxicity Study of TAK-375 in Rats (continued)

Daily Dose (mg/kg)	0 (Control)		2.5		10		40		150	
	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
Hematology										
RBC ($\times 10^4/\mu\text{L}$)	-	830	-	818	-	848	-	821	-	784*
Hematocrit (%)	47.8	45.7	46.2	45.0	46.5	46.1	47.9	45.6	45.7*	42.8**
Hemoglobin (g/dL)	16.6	16.0	15.8*	15.7	16.1	16.1	16.4	15.9	15.8**	14.7**
Clinical Chemistry										
Total protein (g/dL)	6.46	6.92	6.37	6.66	6.43	6.75	6.51	7.00	6.82**	7.54**
Total cholesterol (mg/dL)	-	97	-	99	-	102	-	103	-	164**
Organ Weights (%) (a)										
Liver (g)	13.08	6.63	-5.0	+1.1	-2.4	-2.3	+2.4	+5.7	+12.2*	+43.1**
Kidneys (g)	-	1.70	-	+0.6	-	+0.6	-	+5.3	-	+10.6**
Spleen (g)	-	0.43	-	+7.0	-	+4.7	-	+7.0	-	+16.3*
Adrenal gland (mg)	-	57.7	-	-4.0	-	-6.8	-	+0.3	-	+15.3*
Thyroid gland (mg)	23.1	20.1	+8.2	-2.0	+10.0	-2.0	+19.0**	+3.5	+26.0**	+16.4*
Gross Pathology										
Dark discolored liver	-	0	-	0	-	1	-	0	-	8
Enlarged liver	-	0	-	0	-	0	-	0	-	4
Histopathology										
Liver:										
Hepatocellular hypertrophy	0	0	0	0	0	0	5	4	14	15
Minimal	0	0	0	0	0	0	4	4	7	5
Mild	0	0	0	0	0	0	1	0	7	10
Adrenal gland:										
Vacuolization of cortical cells	0	0	0	0	0	0	0	1	8	3
Minimal	0	0	0	0	0	0	0	1	8	3

APPENDIX 4 (cont.)

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2.6.7.7C Repeat-Dose Toxicity: Twenty-Six Week Oral Gavage Toxicity Study of TAK-375 in Rats

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2.6.7.7C Repeat-Dose Toxicity: Twenty-Six-Week Oral Gavage Toxicity Study of TAK-375 in Rats (continued)

Daily Dose (mg/kg)	0 (Control)		2.5		10		40		150	
Number of Animals	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15	M: 15	F: 15
Thyroid gland:										
Hypertrophy of follicular cells	0	0	0	0	1	0	1	0	5	6
Minimal	0	0	0	0	1	0	1	0	5	6
Ovaries:										
Vacuolization of interstitial cells	NA	0	NA	0	NA	0	NA	0	NA	12
Minimal		0		0		0		0		4
Mild		0		0		0		0		8
Spleen:										
Hematopoietic cell proliferation	13	14	14	14	13	14	15	15	13	14
Minimal	13	13	14	11	13	14	15	14	13	9
Mild	0	1	0	3	0	0	0	1	0	5
Pigmentation	15	15	15	15	15	15	15	15	15	15
Minimal	15	15	15	15	15	15	15	14	15	7
Mild	0	0	0	0	0	0	0	1	0	8
Additional Examinations										
T3 (ng/mL); (N: 8)	-	1.11	-	1.13	-	1.11	-	1.09	-	0.96**
T4 (µg/dL); (N: 8)	-	-	-	-	-	-	-	-	-	-
TSH (ng/mL); (N: 8)	-	0.31	-	0.46	-	0.30	-	0.45	-	0.69*
Aminopyrine-N-demethylase; (N: 5) (mU/mg protein)	-	-	-	-	-	-	-	-	-	-
Aniline hydroxylase (N: 5) (mU/mg protein)	-	-	-	-	-	-	-	-	-	-

- indicates no noteworthy findings; Dunnett's Test: *, $p \leq 0.05$; **, $p \leq 0.01$; NA, not applicable; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone.

(a) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

2.6.7.7D Repeat-Dose Toxicity: Four-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys

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2.6.7.7D Repeat-Dose Toxicity: Four-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys

Species/Strain:	Cynomolgus Monkeys	Duration of Dosing:	4 Weeks	Test Article	TAK-375
Initial Age:	2 years 11 months to 3 years 2 months	Duration of Postdose:	None	Study/Report No.:	2700/SU/M-11-26-1
Date of First Dose:	30 Mar 98	Method of Administration:	Gavage	GLP Compliance:	Yes
				Vehicle/Formulation:	0.5 w/v% methylcellulose solution

Special Features: Plasma T3, T4 levels; activity of hepatic drug-metabolizing enzymes (aniline hydroxylase and aminopyrine-N-demethylase).

No Observed Adverse Effect Level: 12 mg/kg/day

Daily Dose (mg/kg)	0 (Control)		3		12		50		200	
	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Toxicokinetics										
C _{max} (µg/mL)	NA	NA	0.05	0.01	0.73	0.44	2.73	2.55	9.28	13.12
AUC (µg·hr/mL)	NA	NA	0.14	0.04	1.23	0.87	9.06	10.36	75.29	86.73
Noteworthy Findings										
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0	0	0
Body Weight (%)	-	-	-	-	-	-	-	-	-	-
Food Consumption (%)	-	-	-	-	-	-	-	-	-	-
Water Consumption	-	-	-	-	-	-	-	-	-	-
Body Temperature										
Day 0	-	-	-	-	↓(1)	↓(1)	↓(1)	↓(1)	↓(2)	↓(3)*
Heart rate										
Day 0	-	-	-	-	↓(1)	-	↓(1)	-	↓(1)	↓(1)
Clinical Observations										
Vomiting	-	-	-	-	-	-	1	1	3	3
Ophthalmoscopy										
Hematology	-	-	-	-	-	-	-	-	-	-
Clinical Chemistry										
ALT (U/L)	37	31	24	31	36	31	30	37	57 ^b	99 ^b

APPENDIX 4 (cont.)

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2.6.7.7D Repeat-Dose Toxicity: Four-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys

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2.6.7.7D Repeat-Dose Toxicity: Four-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys (continued)

Daily Dose (mg/kg)	0 (Control)		3		12		50		200	
Number of Animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Urinalysis	-	-	-	-	-	-	-	-	-	-
Organ Weights (%) (a)										
Liver	53.1 g	58.3 g	-2.8	+10.8	+15.8	+8.9	+17.7	+5.8	+50.5*	+35.8**
Gross Pathology	-	-	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-	-	-
Additional Examinations										
T3 (ng/mL)	-	-	-	-	-	-	-	-	-	-
T4 (µg/dL)	-	-	-	-	-	-	-	-	-	-
Aniline hydroxylase (mU/mg protein)	0.100	0.116	0.099	0.115	0.117	0.099	0.160	0.125	0.163	0.172
Aminopyrine-N-demethylase (mU/mg protein)	0.881	0.882	0.876	1.019	0.953	1.113	1.127	1.272	1.281	1.536**

- indicates no noteworthy findings; Dunnett's Test: *, $p \leq 0.05$; **, $p \leq 0.01$; ↓, decreased; NA, not applicable; T3, triiodothyronine; T4, thyroxine; ALT, alanine aminotransferase.

Numbers in parentheses indicate number of affected animals.

(a) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(b) Includes 1 male with an ALT of 92 U/L and 1 female with an ALT of 209 U/L.

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2.6.7.7E Repeat-Dose Toxicity: 39-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys

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2.6.7.7E Repeat-Dose Toxicity: 39-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys (continued)

Daily Dose (mg/kg)	0 (Control)		3		12		50		200	
	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Clinical Observations										
Emesis	-	-	-	-	-	-	-	1	1	3
Foamy material in cage	-	-	-	-	-	-	-	-	4	4
Decreased activity	-	-	-	-	-	-	1	-	4	4
Convulsions (b)	-	-	-	-	-	-	-	-	2	-
Lying on side	-	-	-	-	-	-	-	-	-	1
Body temperature	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-	-	-
Clinical Chemistry										
ALT (U/L)										
Week 13	36.0	-	57.8	-	49.0	-	61.8	-	104.5**	-
Week 39	43.8	71.5	54.0	72.0	40.5	59.3	47.0	54.0	238.8 [#]	124.8
Triglyceride (mg/dL)										
Week 13	30.0	-	34.5	-	42.3	-	39.3	-	79.3**	-
Week 39	28.5	-	31.8	-	34.0	-	44.5	-	69.8**	-
Gross Pathology	-	-	-	-	-	-	-	-	-	-
Organ Weights (%) (a)										
Liver (g)	73.021	62.036	-2.3	-5.0	+6.2	-2.1	+14.3	+3.4	+67.7	+43.2

2.6.7.7E Repeat-Dose Toxicity: 39-Week Oral Gavage Toxicity Study of TAK-375 in Monkeys (continued)

Daily Dose (mg/kg)	0 (Control)		3		12		50		200	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Histopathology										
Liver										
Hepatocellular hypertrophy	0	0	0	0	0	0	0	0	4	4
Minimal	0	0	0	0	0	0	0	0	2	1
Slight	0	0	0	0	0	0	0	0	2	3
Additional Examinations										
Activity of hepatic drug-metabolizing enzymes	-	-	-	-	-	-	-	-	-	-

- indicates no noteworthy findings; Dunnett's test: **, $p < 0.01$; Dunn's test: #, $p < 0.05$; <LLQ, below lower limit of quantitation; NA, not applicable.

(a) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(b) Male 501 convulsed post dose on Day 135 and Male 504 convulsed post dose on Day 69.

2.6.7.7F Repeat-Dose Toxicity: Four-Week Intravenous Toxicity Study of M-II (2*S*,8*S*)
(T-163302), a Metabolite of TAK-375 in Monkeys (continued)

Daily Dose (mg/kg)	0 (Control)		1		3		10	
Number of Animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Gross Pathology	-	-	-	-	-	-	-	-
Histopathology	-	-	-	-	-	-	-	-
Liver drug metabolizing enzymes	-	-	-	-	-	-	-	-

- indicates no noteworthy findings; NA, not applicable; IV, intravenous injection.

(a) M-II (2*S*,8*S*) is a metabolite of TAK-375. The epimer (2*R*,8*S*) was not detected in any sampling points (quantitation limit: ●ng/mL).

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2.6.7.7G Repeat-Dose Toxicity: Thirteen-Week Oral Gavage Range-Finding Study of TAK-375 in Mice

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2.6.7.7G Repeat-Dose Toxicity: Thirteen-Week Oral Gavage Range-Finding Toxicity Study of TAK-375 in Mice (continued)

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M: 12	F: 12	M: 12	F: 12	M: 12	F: 12	M: 12	F: 12	M: 12	F: 12
Adrenal gland:										
Increased fine vacuole (zona fasciculata)	0	0	0	0	0	0	0	0	4	0
Slight	0	0	0	0	0	0	0	0	4	0
Increased large vacuole (zona fasciculata)	0	0	0	0	0	0	0	0	0	5
Slight	0	0	0	0	0	0	0	0	0	5
Hypertrophy of the cortical cells in zona fasciculata	0	0	0	0	0	0	0	0	0	4
Slight	0	0	0	0	0	0	0	0	0	4
Additional Examinations										
Aniline hydroxylase (N: 5) (n mole/mg protein/min)	-	-	-	-	-	-	-	-	-	-
Aminopyrine-N-demethylase (N: 5) (n mole/mg protein/min)	0.601	0.791	0.668	0.914	0.737*	1.070**	0.745*	1.245**	0.887**	1.280**

- indicates no noteworthy findings; NA, not applicable; Dunnett's Test: *, p<0.05; **, p<0.01; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

(a) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

2.6.7.7H Repeat-Dose Toxicity: Thirteen-Week Oral Gavage Toxicity Study of TAK-375 in Rats

Species/Strain:	Jcl:Wistar Rats	Duration of Dosing:	13 Weeks	Test Article:	TAK-375					
Initial Age:	6 Weeks	Duration of Postdose:	None	Study/Report No.:	2934/CA/M-11-00171.001A					
Date of First Dose:	3 Feb 99	Method of Administration:	Gavage	GLP Compliance:	Yes					
		Vehicle/Formulation:	0.5 w/v% methylcellulose solution							
Special Features:	Plasma T3, T4 and TSH levels; activity of hepatic drug-metabolizing enzymes (aniline hydroxylase and aminopyrine-N-demethylase)									
No Observed Adverse Effect Level:	<40 mg/kg/day									
Daily Dose (mg/kg)	0 Control		40		150		600		1200	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Toxicokinetics (N: 3)										
TAK-375 Cmax (µg/mL)	NA	NA	0.673	1.510	1.932	7.958	3.847	9.866	7.860	14.778
M-I Cmax (µg/mL)	NA	NA	0.938	0.721	2.048	2.378	5.569	5.133	10.067	7.326
M-II Cmax (µg/mL)	NA	NA	0.131	0.733	0.268	1.802	0.355	1.205	0.553	1.497
M-III Cmax (µg/mL)	NA	NA	0.407	0.749	1.016	3.124	2.242	5.402	4.779	9.638
M-IV Cmax (µg/mL)	NA	NA	0.035	0.100	0.058	0.285	0.085	0.277	0.168	0.421
TAK-375 AUC (µg·hr/mL)	NA	NA	1.154	2.774	7.724	12.353	14.251	41.611	41.242	175.373
M-I AUC (µg·hr/mL)	NA	NA	2.096	1.797	9.992	6.205	23.408	24.728	64.548	66.321
M-II AUC (µg·hr/mL)	NA	NA	0.390	2.890	1.689	7.248	1.730	6.823	3.604	14.215
M-III AUC (µg·hr/mL)	NA	NA	1.304	3.128	6.229	12.088	14.619	41.420	43.306	137.406
M-IV AUC (µg·hr/mL)	NA	NA	0.222	1.149	0.551	1.959	0.751	2.630	1.784	5.643
Noteworthy Findings										
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0	0	0
Body Weight (%) (a)										
Week 13	444 g	268 g	-7.9**	-4.1	-8.6**	-8.2**	-7.4*	-11.2**	-16.9**	-0.7
Food Consumption (%) (a)										
Week 13	-	110 g	-	+0.9	-	-8.2	-	-0.9	-	+16.4*

APPENDIX 4 (cont.)

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2.6.7.7H Repeat-Dose Toxicity: Thirteen-Week Oral Gavage Toxicity Study of TAK-375 in Rats

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2.6.7.7H Repeat-Dose Toxicity: Thirteen-Week Oral Gavage Toxicity Study of TAK-375 in Rats (continued)

Daily Dose (mg/kg)	0 (Control)		40		150		600		1200	
	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Clinical Observations										
Salivation	-	-	-	-	-	-	1	-	8	5
Chromaturia	-	-	-	-	-	-	-	-	6	3
Licking	-	-	-	-	-	-	-	-	1	2
Decreased locomotor activity	-	-	-	-	-	-	-	1	-	1
Distended abdomen	-	-	-	-	-	-	-	-	-	3
Hematology										
Platelet (x10 ⁴ /μL)	104.7	107.7	97.3	103.5	102.8	114.2	117.4*	114.0	131.5**	132.4**
Reticulocytes (%)	2.6	3.2	2.5	3.2	2.8	3.2	3.0**	3.5	3.4**	4.1**
Clinical Chemistry										
Total protein (g/dL)	6.30	5.96	6.12	5.64*	6.35	6.07	6.71**	6.42**	6.74**	7.01**
A/G ratio	1.13	1.37	1.20*	1.46*	1.18	1.29	1.12	1.20**	0.99**	1.04**
Total cholesterol (mg/dL)	71	62	83	63	83	79	97**	127**	128**	161**
Triglyceride (mg/dL)	137	71	133	64	129	87	174	116*	311**	173**
Calcium (mg/dL)	9.16	9.07	9.17	9.00	9.43*	9.21	9.92**	9.87**	10.22**	10.58**
Organ Weights (%) (a)										
Liver (g)	11.55	6.16	-4.9	-2.3	+4.9	+16.7	+57.0**	+85.6**	+92.5**	+220.8**
Kidneys (g)	2.54	1.63	-6.3	-3.1	-3.9	-0.6	+9.8*	+1.2	+7.5	+22.1**
Thyroids (mg)	18.9	15.0	+4.2	+5.3	+2.6	+8.0	+31.2**	+20.7*	+37.0**	+66.7**
Adrenals (mg)	57.5	66.6	-7.7	-6.5	-4.7	-0.2	+13.0	+37.4*	+66.3**	+175.8**
Ovaries (mg)	NA	93.4	NA	-0.4	NA	-2.4	NA	+33.4	NA	+102.7**
Thymus (mg)	191.4	201.0	+28.4*	+4.9	+27.7*	+0.9	-7.3	-1.3	-26.8*	-28.7**
Carcass (g)	337	205	-6.8*	-5.4*	-9.5**	-9.3**	-11.0**	-18.0**	-26.1**	-17.6**

2.6.7.7H Repeat-Dose Toxicity: Thirteen-Week Oral Gavage Toxicity Study of TAK-375 in Rats (continued)

Daily Dose (mg/kg)	0 (Control)		40		150		600		1200	
	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Number of Animals										
Additional Examinations										
Number examined	5	5	5	5	5	5	5	5	5	5
T3 (ng/mL)	-	1.44	-	1.57	-	1.33	-	1.16**	-	1.14**
T4 (µg/dL)	-	-	-	-	-	-	-	-	-	-
TSH (ng/mL)	0.43	0.16	1.00	0.33	0.68	0.49	2.19**	1.21**	1.44*	1.27**
Number examined	5	5	5	5	5	5	5	5	5	5
Aminopyrine-N-demethylase (mU/mg protein)	-	0.589	-	0.708	-	0.979**	-	1.178**	-	0.890**
Aniline hydroxylase (mU/mg protein)	-	0.043	-	0.058	-	0.088**	-	0.062*	-	0.030

- indicates no noteworthy findings; Dunnett's Test: *, $p \leq 0.05$; **, $p \leq 0.01$; NA, not applicable; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone.

(a) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

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APPENDIX 4 (cont.)

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2.6.7.II Repeat-Dose Toxicity: Four-Week Oral Gavage Toxicity Study of TAK-375 (Lot No. M375.017) in Rats

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2.6.7.II Repeat-Dose Toxicity: Four-Week Oral Gavage Toxicity Study of TAK-375 (Lot No. M375-017) in Rats

Species/Strain	CD(SD)IGS Rats	Duration of Dosing	4 Weeks	Test Article	TAK-375
Initial Age	6 Weeks	Duration of Postdose	None	Study/Report No.	01-392/SU/M-11-00356.001
Date of First Dose	25 Sep 01	Method of Administration	Gavage	GLP Compliance	Yes
Special Features	Lot No. M375-017 [TAK-375 \ contains \ of the \ mpurity.			Vehicle/Formulation	0.5 w/v% methylcellulose solution
Conclusion	No significant differences in toxicity between the TAK-375 \ and TAK-375.				

Test article	Control		TAK-375 150		TAK-375 150	
	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Daily Dose (mg/kg)	0		150		150	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Noteworthy Findings						
Died or Sacrificed Moribund	0	0	0	0	0	0
Body Weight	-	-	-	-	-	-
Food Consumption	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-
Water Consumption (n: 5)	-	-	-	-	-	-
Urine output (n: 5)	-	-	-	-	-	-
Urinalysis (n: 5)	-	-	-	-	-	-
Clinical Observations						
Salivation	0	0	4	1	6	5
Hematology						
	-	-	-	-	-	-
Clinical Chemistry						
Total protein (g/dL)	6.0	6.2	6.2**	6.4*	6.2**	6.4
Total cholesterol (mg/dL)	-	83	-	118**	-	119**
Triglyceride (mg/dL)	54	38	37*	47	44	66**

2.6.7.71 Repeat-Dose Toxicity: Four-Week Oral Gavage Toxicity Study of TAK-375 (Lot No. M375-017) in Rats (continued)

Test article	Control		TAK-375 \		TAK-375	
	0		150		150	
Daily Dose (mg/kg)						
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Noteworthy Findings						
Organ Weights (%) (a)						
Liver (g)	9.85	5.91	+10.4	+18.1**	+9.8*	+16.4*
Pituitary (mg)	11.4	-	-4.4	-	-15.8**	-
Thymus (mg)	-	389.2	-	-1.7	-	-15.4**
Gross Pathology						
Enlarged liver	0	0	3	0	2	0
Dark discoloration- liver	0	0	0	1	0	2
Histopathology						
Liver:						
Hepatocellular hypertrophy	0	0	4	5	6	8
Minimal	0	0	4	5	6	8
Ovary:						
Vacuolization of interstitial cell	NA	0	NA	6	NA	6
Minimal		0		6		6

- indicates no noteworthy findings; Dunnett's Test: *, $p \leq 0.05$; **, $p \leq 0.01$; NA, not applicable.

(a) For controls, group means are shown. For treated groups, percent differences from the controls are shown. Statistical significance is based on the actual data (not the percent differences).

APPENDIX 4 (cont.)

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2.6.7.8A Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375

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2.6.7.8A Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375

Test for Induction of:	Reverse mutation in bacterial cells	No. of Independent Assays:	2	Test Article:	TAK-375
Strains:	S. typhimurium and E coli	No. of Replicate Cultures:	3 (solvent control); 2 (test article and positive control)	Study/Report No.:	3643/M-11-13
Metabolizing System:	Phenobarbital and 5, 6-benzoflavone-induced rat liver S9; 7.1%	No. of Cells Analyzed/Cultures:	-	GLP Compliance:	Yes
Vehicles:	For Test Article:	DMSO	For Positive Controls:	DMSO	
Treatment:	Preincubation for 20 minutes followed by incubation for 48 hours				
Cytotoxic Effects:	None				
Genotoxic Effects:	None				
Date of Treatment: November 1997					

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay No. 1 Revertant Colony Counts (Mean)				
			TA100	TA1535	WP2uvrA	TA98	TA1537
Without Activation	DMSO	0	128	15	16	31	9
	TAK-375	51.2	136	11	18	31	9
		128	124	11	15	32	8
		320	123	15	15	33	8
		800	119	12	15	27	7
		2000	117	14	20	36	8
		5000 (a)	101	4	8	27	1
	2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.01	466	-	117	-	-
		0.1	-	-	-	595	-
		0.5	-	480	-	-	-
9-aminoacridine hydrochloride	80	-	-	-	-	420	
	DMSO	0	127	10	16	35	14
With Activation	TAK-375	51.2	124	13	21	41	16
		128	135	11	22	37	10
		320	120	13	17	38	13
		800	126	10	23	30	12
		2000	127	12	16	46	9
		5000 (a)	129	6	24	38	5

2.6.7.8A Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375 (continued)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay No. 2 Revertant Colony Counts (Mean)				
			TA100	TA1535	WP2uvrA	TA98	TA1537
With Activation	2-Aminoanthracene	0.5	-	-	-	175	-
		1	730	-	-	-	-
		2	-	250	-	-	127
		10	-	-	615	-	-
Without Activation	DMSO	0	119	12	15	19	8
	TAK-375	156	107	12	17	19	8
		313	107	12	16	20	6
		625	117	12	14	17	7
		1250	114	11	17	21	10
		2500	94	12	12	21	9
		5000 (a)	104	8	13	17	5
	2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.01	414	-	115	-	-
		0.1	-	-	-	485	-
		0.5	-	335	-	-	-
	9-Aminoacridine hydrochloride	80	-	-	-	-	506
	With Activation	DMSO	0	125	10	21	27
TAK-375		156	135	9	21	25	15
		313	115	12	20	27	14
		625	116	13	22	27	17
		1250	116	11	20	23	16
		2500	129	12	24	26	10
		5000 (a)	111	8	19	24	6
2-Aminoanthracene		0.5	-	-	-	162	-
		1	686	-	-	-	-
		2	-	275	-	-	115
		10	-	-	662	-	-

(a) Precipitation at the end of the exposure period.

APPENDIX 4 (cont.)

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2.6.7.8B Genotoxicity: In Vitro Report Title: Mammalian Cell Gene Mutation Test

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2.6.7.8B Genotoxicity: In Vitro Report Title: Mammalian Cell Gene Mutation Test

Test for Induction of:	Gene mutation	No. of Independent Assays:	2	Test Article:	TAK-375
Strains:	L5178Y mouse lymphoma cells	No. of Replicate Cultures:	2 (1 for positive controls)	Study/Report No.:	G97CB86.702/M-11-19-1
Metabolizing System:	Aroclor-1254 induced rat liver S9, 10% Aroclor-1254 induced rat liver S9, 10%	No. of Cells Analyzed/Cultures:	10 ⁶ clonable cells	GLP Compliance:	Yes
Vehicles:	For Test Article: DMSO	For Positive Controls:	-	Date of Treatment:	November, 1997
Treatment:	Four hours in the presence and absence of S9 activation				
Cytotoxic Effects:	Dose-related decreases in cell growth				
Genotoxic Effects:	None				

Metabolic Activation	Test Article	Concentration (µg/mL)	Cytotoxicity (% of control) (a)		Cytotoxicity (% of control) (b)		Mutant Frequency (/10 ⁶ surviving cells)			
			A	B	A	B	A	B		
Assay No. 1										
Without Activation	DMSO	0	-	-	-	-	70	78		
		100	97	92	81	74	86	87		
		150	77	69	72	53	81	73		
		200	68	65	57	54	94	88		
		300	37	41	37	39	64	65		
		400	18	20	16	17	87	97		
	Methyl methanesulfonate	10	75	-	57	-	266	-		
		20	48	-	18	-	350	-		
		With Activation	DMSO	0	-	-	-	-	103	79
				100	99	101	105	89	70	98
150	80			86	90	93	(c)	79		
200	78			74	87	78	79	92		
300	38			35	37	39	102	79		
400	12			10	14	12	90	81		
7, 12-Dimethyl benz(a)anthracene	2.5	104	-	94	-	77	-			
	4.0	28	-	14	-	537	-			

2.6.7.8B Genotoxicity: In Vitro Report Title: Mammalian Cell Gene Mutation Test (continued)

Metabolic Activation	Test Article	Concentration ($\mu\text{g/mL}$)	Cytotoxicity (% of control) (a)		Cytotoxicity (% of control) (b)		Mutant Frequency (/10 ⁶ surviving cells)	
			A	B	A	B	A	B
Assay No. 2								
Without Activation	DMSO	0	-	-	-	-	46	39
	TAK-375	100	76	81	77	91	56	70
		200	51	47	56	49	60	69
		300	24	19	21	19	80	84
		400	11	9	8	6	(d)	(d)
		500	27	24	30	23	73	75
	Methyl methanesulfonate	10	57	-	37	-	423	-
		20	31	-	3	-	1121	-
With Activation	DMSO	0	-	-	-	-	58	38
	TAK-375	50	107	102	120	108	51	64
		100	107	107	128	120	64	66
		150	82	77	88	89	81	67
		200	58	57	61	61	65	66
		300	17	16	18	15	69	64
	7, 12 Dimethyl benz(a)anthracene	2.5	79	-	38	-	246	-
		4.0	27	-	14	-	517	-

(a) Based on suspension growth.

(b) Based on % total growth.

(c) Culture lost.

(d) Total growth <10%.

A and B are duplicate cultures.

APPENDIX 4 (cont.)

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2.6.7.8C Genotoxicity: In Vitro Report Title: Chromosomal Aberration
Test on Chinese Hamster Lung Cells Treated with TAK-375

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2.6.7.8C Genotoxicity: In Vitro Report Title: Chromosomal Aberration Test on Chinese Hamster Lung Cells Treated with TAK-375

Test for Induction of:	Chromosome aberration	No. of Independent Assays:	1	Test Article:	TAK-375
Strains:	Chinese hamster lung cells	No. of Replicate Cultures:	2	Study/Report No.:	3644/M-11-17-1
Metabolizing System:	Phenobarbital and 5, 6-benzoflavone-induced rat liver S9 5%	No. of Cells Analyzed/Cultures:	100	GLP Compliance:	Yes
Vehicles:	For Test Article:	DMSO	For Positive Controls:	Saline	
Treatment:	Continuous treatment for 24 and 48 hours without S9; pulse treatment for 6 hours with 18-hour recovery time with and without S9				
Cytotoxic Effects:	Dose-related decreases in cell survival				
Genotoxic Effects:	Chromosome aberrations at $\geq 113 \mu\text{g/mL}$ (48 hour treatment) and at $450 \mu\text{g/mL}$ with S9, concurrent with decreased cell survival rate and/or decreased mitotic indices.				

Metabolic Activation	Test Article	Exposure-		Cytotoxicity (% of control) (a)	Cytotoxicity (% of control) (b)	Aberrant Cells Mean%	Polyploid Cells % (c)	Judgement (d)
		Concentration ($\mu\text{g/mL}$)	Expression Time (hr)					
Without Activation	DMSO	0	24-0	100	100	0.0	1.0	-
	TAK-375	56.3	24-0	97.6	76.7	1.5	2.1	-
		113	24-0	90.7	63.3	3.5	1.2	-
		225	24-0	67.6	26.7	4.0	2.2	-
		450 (e)	24-0	7.9	0.0	ND	ND	NA
	Mitomycin C	0.05	24-0	ND	ND	43.0	ND	+
Without Activation	DMSO	0	48-0	100	100	1.0	0.9	-
	TAK-375	56.3	48-0	89.1	73.0	2.0	2.0	-
		113	48-0	65.8	73.0	5.5	3.3	$\pm$
		225	48-0	42.2	20.6	9.0	2.7 (f)	$\pm$
		450 (e)	48-0	5.0	0.0	ND	ND	NA
	Mitomycin C	0.025	48-0	ND	ND	40.0	ND	+

2.6.7.8C Genotoxicity: In Vitro Report Title: Chromosomal Aberration Test on Chinese Hamster Lung Cells
Treated with TAK-375 (continued)

Metabolic Activation	Test Article	Concentration (µg/mL)	Exposure-Expression Time (hr)	Cytotoxicity (% of control) (a)	Cytotoxicity (% of control) (b)	Aberrant Cells Mean%	Polyploid Cells % (c)	Judgement (d)	
Without Activation	DMSO	0	6-18	100	100	0.5	1.0	-	
		TAK-375	56.3	6-18	97.8	80.4	0.5	1.5	-
			113	6-18	100.2	94.1	1.0	2.1	-
			225	6-18	82.0	98.0	0.0	1.4	-
			450 (e)	6-18	9.5	7.8	ND	ND	NA
		Cyclophosphamide	12.5	6-18	ND	ND	0.0	ND	-
With Activation	DMSO	0	6-18	100	100	0.5	0.8	-	
		TAK-375	113	6-18	93.6	107.2	0.0	0.9	-
			225	6-18	86.8	116.9	0.5	0.8	-
			450 (e)	6-18	50.9	63.9	11.5	3.5	+
			900 (e)	6-18	10.4	4.8	ND	ND	NA
		Cyclophosphamide	12.5	6-18	ND	ND	39.5	ND	+

NA indicates Not applicable; ND, Not determined.

(a) Based on cell survival.

(b) Based on mitotic indices.

(c) Based on 1000 metaphases.

(d) Judgement was made according to the total (%) of structural aberrations and polyploid cells. -: negative (both values are <5%). ±: inconclusive (either of the values is ≥5% and <10%). +: positive (either of the values is ≥10%).

(e) Precipitation at the end of treatment.

(f) Based on 926 metaphases.

APPENDIX 4 (cont.)

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2.6.7.8D Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375 (Lot No. M375-010)

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2.6.7.8D Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375 (Lot No. M375-010)

Test for Induction of:	Reverse mutation in bacterial cells	No. of Independent Assays:	2	Test Article:	TAK-375
Strains:	S. typhimurium and E. coli	No. of Replicate Cultures:	4 (solvent control); 2 (test article and positive control)	Study/Report No.:	2765/GE/M-11-52
Metabolizing System	Phenobarbital and 5, 6-benzoflavone-induced rat liver S9; 7.1%	No. of Cells Analyzed/Cultures:	-	GLP Compliance:	Yes
Vehicles:	For Test Article: DMSO	For Positive Controls:	DMSO	Date of Treatment:	July/August, 1998
Treatment:	Preincubation for 20 minutes followed by incubation for 48 hours				
Cytotoxic Effects:	Without S9: growth inhibition at ≥ 2000 $\mu\text{g}/\text{plate}$ with TA100, TA1535, and TA1537. With S9: growth inhibition at 5000 $\mu\text{g}/\text{plate}$ with TA100 and TA1535 and at ≥ 2000 $\mu\text{g}/\text{plate}$ with TA1537.				
Genotoxic Effects:	None				

Metabolic Activation	Test Article	Dose Level ($\mu\text{g}/\text{plate}$)	Revertant Colony Counts (Mean)					
			TA100	TA1535	WP2uvrA	TA98	TA1537	
Assay No. 1 Without Activation	DMSO	0	148	11	18	18	9	
	TAK-375	51.2	147	15	19	20	10	
		128	162	9	18	24	7	
		320	154	11	26	29	11	
		800	143	8	18	22	8	
		2000	102 (b)	5 (b)	15	19	1 (b)	
		5000 (a)	160 (b)	7 (b)	13	21	1 (b)	
		2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.01	506	-	98	-	-
	Sodium azide	0.1	-	-	-	408	-	
	9-Aminoacridine hydrochloride	0.5	-	464	-	-	-	
	80	-	-	-	-	301	-	
	With Activation	DMSO	0	106	12	20	34	17
		TAK-375	51.2	120	11	23	34	21
128			129	19	25	25	17	
320			118	8	24	35	11	
800			99	8	19	32	14	
2000			92	9	23	36	5 (b)	
5000 (a)			42 (b)	4 (b)	21	38	4 (b)	
2-Aminoanthracene			1	584	-	-	240	-
2		-	270	-	-	-	115	
10		-	-	496	-	-	-	

2.6.7.8D Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375 (Lot No. M375-010) (continued)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean)					
			TA100	TA1535	WP2uvrA	TA98	TA1537	
Assay No. 2 Without Activation	DMSO	0	139	12	19	26	6	
	TAK-375	156	144	15	20	22	4	
		313	136	17	13	29	4	
		625	133	18	26	23	9	
		1250	123	11	20	18	6	
		2500 (a)	77 (b)	11 (b)	22	18	4 (b)	
		5000 (a)	20 (b)	6 (b)	13	21	2 (b)	
	2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.01	495	-	86	-	-	
		0.1	-	-	-	320	-	
		0.5	-	499	-	-	-	
	9-Aminoacridine hydrochloride	80	-	-	-	-	433	
	With Activation	DMSO	0	108	17	25	31	16
		TAK-375	156	97	17	24	27	12
313			92	12	30	30	16	
625			100	18	23	36	12	
1250			89	17	32	23	10	
2500 (a)			86	10	25	29	7 (b)	
5000 (a)			40 (b)	1 (b)	26	31	2 (b)	
2-Aminoanthracene		1	628	-	-	294	-	
		2	-	356	-	-	127	
		10	-	-	502	-	-	

(a) Precipitation at the end of the exposure period.

(b) Growth inhibition.

APPENDIX 4 (cont.)

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2.6.7.8E Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375 (Lot No. M375-017)

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2.6.7.8E Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375 (Lot No. M375-017)

Test for Induction of Strains:	Reverse mutation in bacterial cells S. typhimurium and E coli	No. of Independent Assays: 2	No. of Replicate Cultures: 3 (solvent control); 2 (test article and positive control)	Test Article: TAK-375	Study/Report No.: SR01048/M-11-00353
Metabolizing System:	Phenobarbital and 5, 6-benzoflavone- induced rat liver S9; 7,1%	No. of Cells Analyzed/Cultures: -		GLP Compliance: Yes	Date of Treatment: October/November, 2001
Vehicles:	For Test Article: DMSO	For Positive Controls: DMSO [2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide, 9-aminoacridine hydrochloride, and 2-aminoanthracene]; water (sodium azide)	Cytotoxic Effects: None	Genotoxic Effects: None	
Treatment:	Preincubation for 20 minutes followed by incubation for 48 hours				

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean)				
			TA100	TA1535	WP2uvrA	TA98	TA1537
Assay No. 1 Without Activation	DMSO	0	111	8	15	15	12
	TAK-375	51.2	113	7	10	15	8
		128	109	6	18	19	11
		320	120	7	15	14	11
		800	109	12	13	20	14
		2000	98	7	12	18	10
		5000	61	6	12	15	11
	2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.01	1126	-	163	-	-
		0.1	-	-	-	543	-
		Sodium azide	0.5	-	271	-	-
	9-Aminoacridine hydrochloride hydrate	80	-	-	-	-	278

2.6.7.8E Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375 (Lot No. M375-017) (continued)

Metabolic Activation	Test Article	Dose Level ($\mu\text{g}/\text{plate}$)	Revertant Colony Counts (Mean)					
			TA100	TA1535	WP2uvrA	TA98	TA1537	
Assay No. 1 With Activation	DMSO	0	125	6	20	29	16	
		TAK-375	51.2	120	8	22	32	16
			128	147	10	20	30	17
			320	125	7	19	28	15
			800	133	6	17	32	17
			2000	104	5	19	31	9
	5000	59	3	19	24	3		
	2-Aminoanthracene	0.5	-	-	-	202	-	
		1	1238	-	-	-	-	
		2	-	132	-	-	254	
10		-	-	776	-	-		

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2.6.7.8E Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375 (Lot No. M375-017)

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2.6.7.8E Genotoxicity: In Vitro Report Title: Reverse Mutation Assay of TAK-375 (Lot No. M375-017) (continued)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean)				
			TA100	TA1535	WP2uvrA	TA98	TA1537
Assay No. 2 Without Activation	DMSO	0	112	10	20	12	10
	TAK-375	156	104	11	19	13	8
		313	116	7	20	15	9
		625	120	8	18	15	10
		1250	103	10	24	12	10
		2500	58	5	11	13	9
		5000	55	7	9	13	8
	2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.01	1180	-	174	-	-
		0.1	-	-	-	239	-
		0.5	-	170	-	-	-
	Sodium azide	0.5	-	-	-	-	-
		9-Aminoacridine hydrochloride	80	-	-	-	306
	With Activation	DMSO	0	146	12	23	27
TAK-375		156	143	15	23	22	23
		313	125	16	21	29	15
		625	154	13	21	34	13
		1250	132	12	25	22	9
		2500	98	9	18	28	11
		5000	76	7	21	23	5
2-Aminoanthracene		0.5	-	-	-	250	-
		1	1203	-	-	-	-
		2	-	201	-	-	339
	10	-	-	736	-	-	

2.6.7.8F Genotoxicity: In Vitro

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Report Title: Cytogenetic Assay with TAK-375 (Lot No. M375-017) in Chinese Hamster Lung (CHL) Cells

2.6.7.8F Genotoxicity: In Vitro Report Title: Cytogenetic Assay with TAK-375 (Lot No. M375-017) in Chinese Hamster Lung (CHL) Cells

Test for Induction of:	Chromosome aberration	No. of Independent Assays:	1	Test Article:	TAK-375
Strains:	Chinese hamster lung cells	No. of Replicate Cultures:	2	Study/Report No.:	SR01049/M-11-00354
Metabolizing System:	Phenobarbital and 5, 6-benzoflavone-induced rat liver S9 5%	No. of Cells Analyzed/Cultures:	100	GLP Compliance:	Yes
Vehicles:	For Test Article:	DMSO	For Positive Controls:	Saline for mitomycin C; DMSO for benzo[a]pyrene	
Treatment:	Continuous treatment for 24 hours without S9; pulse treatment for 6 hours with 18-hour recovery time with and without S9				
Cytotoxic Effects:	Decreases in growth rate				
Genotoxic Effects:	None				

Metabolic Activation	Test Article	Concentration (µg/mL)	Exposure-Expression Time (hr)	Cytotoxicity (% of control) (a)	Cytotoxicity (% of control) (b)	Aberrant Cells Mean%	Polyploid Cells % (c)	Judgement (d)
Without Activation	DMSO	0	24-0	100	100	2.0	0.4	-
		209.7	24-0	54.5	112.5	0.0	0.0	-
		262.1	24-0	53.5	65.0	1.0	0.0	-
		327.7	24-0	43.0	50.0	0.0	0.1	-
	Mitomycin C	0.05	24-0	ND	ND	50.5	ND	+
Without Activation	TAK-375	0	6-18	100	100	2.5	0.4	-
		262.1	6-18	95.0	128.0	0.5	0.1	-
		327.7	6-18	88.5	154.0	1.0	0.0	-
		409.6	6-18	75.5	110.0	0.5	0.1	-
		512	6-18	17.0	NC	ND	ND	NA
	Mitomycin C	0.1	6-18	ND	ND	43.0	ND	+

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2.6.7.8F Genotoxicity: In Vitro

Report Title: Cytogenetic Assay with TAK-375 (Lot No. M375-017) in Chinese Hamster Lung (CHL) Cells

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2.6.7.8F Genotoxicity: In Vitro Report Title: Cytogenetic Assay with TAK-375 (Lot No. M375-017) in Chinese Hamster Lung (CHL) Cells

Metabolic Activation	Test Article	Concentration (µg/mL)	Exposure-Expression Time (hr)	Cytotoxicity (% of control) (a)	Cytotoxicity (% of control) (b)	Aberrant Cells Mean%	Polyploid Cells % (c)	Judgement (d)
With Activation	DMSO	0	6-18	100	100	1.0	0.1	-
		TAK-375	327.7	6-18	98.5	79.6	2.0	0.0
	Benzo[a]pyrene	409.6	6-18	93.5	86.7	1.0	0.0	-
		512	6-18	99.5	73.5	1.5	0.1	-
		640	6-18	19.0	NC	ND	ND	NA
		10	6-18	ND	ND	36.5	ND	+

NA indicates Not applicable; NC, No observable cells; ND, Not determined; -, negative (both values are <5%); ±, inconclusive (either of the values is ≥5% and <10%); +, positive (either of the values is ≥10%).

(a) Based on growth rate.

(b) Based on mitotic indices.

(c) Based on 1000 metaphases.

(d) Judgment was made according to the total (%) of structural aberrations and polyploid cells.

2.6.7.9A Genotoxicity: In Vivo Report Title: Micronucleus Test on TAK-375 in Mice

Test for Induction of:	Bone marrow micronuclei	Treatment Schedule:	Once daily, 2 days	Test Article:	TAK-375
Species/Strains:	Jel:ICR mice	Sampling Time:	24 hours after last dose	Study/Report No.:	2684/GE/M-11-20
Age:	7 Weeks	Method of Administration:	Gavage (TAK-375 and vehicle); intraperitoneal (mitomycin C)	GLP Compliance:	Yes
Cells Evaluated:	Polychromatic erythrocytes	Vehicle/Formulation:	0.5 w/v% methylcellulose suspension (TAK-375 and vehicle); distilled water (mitomycin C)		
No. of Cells Analyzed/Animal:	2000				
Special Features:	None				
Toxic/Cytotoxic Effects:	250 mg/kg: decreased activity; 500 mg/kg: decreased activity, ataxic gait; 1000 mg/kg: decreased activity, respiratory depression, loss of righting reflex. No cytotoxic effects.				
Genotoxic Effects:	None				
Evidence of Exposure:	None				

Test Article	Daily Dose (mg/kg)	No. of Animals	% Polychromatic Erythrocytes (range)	% Micronucleated Polychromatic Erythrocytes (range)
0.5% Methylcellulose	0	5M	63.0 (59.9-66.4)	0.11 (0.05-0.15)
TAK-375	250	5M	70.6 (65.8-75.6)	0.12 (0.05-0.20)
TAK-375	500	5M	61.5 (50.8-71.5)	0.13 (0.10-0.15)
TAK-375	1000	5M	63.2 (49.1-70.4)	0.13 (0.05-0.20)
Mitomycin C	2	5M	47.3 (34.1-59.7)	6.04 (4.60-7.50)

APPENDIX 4 (cont.)

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2.6.7.9B Genotoxicity: In Vivo Report Title: Micronucleus Test on TAK-375 in Rats

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2.6.7.9B Genotoxicity: In Vivo Report Title: Micronucleus Test on TAK-375 in Rats

Test for Induction of:	Bone marrow micronuclei	Treatment Schedule:	Once daily, 2 days	Test Article:	TAK-375
Species/Strains:	\ CD(SD)IGS rats	Sampling Time:	24 hours after last dose	Study/Report No.:	3645/M-11-18
Age:	7 Weeks	Method of Administration:	Gavage (TAK-375 and vehicle); intravenous (mitomycin C)	GLP Compliance:	Yes
Cells Evaluated:	Immature erythrocytes	Vehicle/Formulation:	0.5 w/v% methylcellulose suspension (TAK-375 and vehicle); distilled water for injection (mitomycin C)		
No. of Cells Analyzed/Animal:	2000				
Special Features:	None				
Toxic/Cytotoxic Effects:	2000 mg/kg: decreased body weight gain. No cytotoxic effects.				
Genotoxic Effects:	None				
Evidence of Exposure:	See Study/Report No. 2699/SU/M-11-00027, Four-Week Oral Gavage Toxicity Study in Rats				

Test Article	Daily Dose (mg/kg)	No. of Animals	Mean % Immature Erythrocytes (±SD)	Mean % Micronucleated Immature Erythrocytes (±SD)
0.5% Methylcellulose	0	5M	64.0±5.2	0.36±0.08
TAK-375	500	5M	68.2±3.5	0.45±0.18
TAK-375	1000	5M	69.9±10.1	0.32±0.08
TAK-375	2000	5M	64.7±3.9	0.36±0.17
Mitomycin C	2	5M	53.9±7.8†	6.85±1.67**

† = p<0.05 (Dunnnett's Test).

** = p<0.01 (Kastenbaum and Bowman).

2.6.7.9C Genotoxicity: In Vivo Report Title: In Vivo/In Vitro Unscheduled DNA Synthesis (UDS)
Assay with TAK-375 in Rat Hepatocytes

Test for Induction of:	Unscheduled DNA synthesis	Treatment Schedule:	Single Dose	Test Article:	TAK-375
Species/Strains:	\ :CD(SD)IGS rats	Sampling Time:	2 and 16 hours	Study/Report No.:	3841/M-11-22
Age:	8 Weeks	Method of Administration:	Gavage	GLP Compliance:	Yes
Cells Evaluated:	Hepatocytes	Vehicle/Formulation:	0.5 w/v% methylcellulose suspension (TAK-375 and vehicle); water for injection (dimethylnitrosamine); corn oil (2-acetylaminofluorene)		
No. of Cells Analyzed/Animal:	150				
Special Features:	None				
Toxic/Cytotoxic Effects:	2000 mg/kg: decreased locomotor activity, nasal discharge, lateral position, decreased body temperature				
Genotoxic Effects:	None				
Evidence of Exposure:	See Study/Report No. 2699/SU /M-11-00027, Four-Week Oral Gavage Toxicity Study in Rats				

Test Article	Daily Dose (mg/kg)	No. of Animals	Time (hours)	Nuclear Mean $\pm$ SD (a)	Cytoplasm Mean $\pm$ SD (b)	NG Mean $\pm$ SD (c)	%IR Mean $\pm$ SD (d)
0.5% Methylcellulose	0	3M	2	6.9 $\pm$ 0.4	8.2 $\pm$ 0.4	-1.3 $\pm$ 0.1	0.0 $\pm$ 0.0
0.5% Methylcellulose	0	3M	16	5.6 $\pm$ 0.2	6.5 $\pm$ 0.5	-0.9 $\pm$ 0.5	1.1 $\pm$ 0.4
TAK-375	1000	3M	2	6.9 $\pm$ 0.7	8.2 $\pm$ 1.7	-1.2 $\pm$ 1.0	2.0 $\pm$ 0.7
TAK-375	1000	3M	16	8.9 $\pm$ 0.9	9.2 $\pm$ 1.3	-0.3 $\pm$ 0.5	2.7 $\pm$ 0.7
TAK-375	2000	3M	2	7.4 $\pm$ 0.1	8.5 $\pm$ 0.6	-1.1 $\pm$ 0.6	3.8 $\pm$ 0.4
TAK-375	2000	3M	16	7.1 $\pm$ 1.1	7.4 $\pm$ 1.3	-0.3 $\pm$ 0.4	4.0 $\pm$ 0.0
Dimethylnitrosamine	5	3M	2	37.1 $\pm$ 2.1	6.5 $\pm$ 1.1	30.6 $\pm$ 1.0	100.0 $\pm$ 0.0
2-Acetylaminofluorene	100	3M	16	37.5 $\pm$ 1.9	8.5 $\pm$ 0.4	29.0 $\pm$ 1.6	100.0 $\pm$ 0.0

(a) Nuclear grain count; the number of grains over the nucleus.

(b) Cytoplasmic grain count; the grain in the nuclear-sized area was counted.

(c) Net grain/nucleus; the nuclear count minus the cytoplasmic count.

(d) Percentage of cells having ≥ 5 nuclear grains.

APPENDIX 4 (cont.)

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2.6.7.10A Carcinogenicity: Twenty-Four-Month Carcinogenicity Study of TAK-375 in Mice

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2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice

Species/Strain:	\ B6C3F1 Mice	Duration of Dosing:	24 Months	Test Article:	TAK-375
Initial Age:	6 Weeks	Method of Administration:	Gavage	Study/Report No:	B-4204/M-11-00560.002A
Date of First Dose:	27 Oct 99 (males) 28 Oct 99 (females)	Vehicle/Formulation:	0.5 w/v% methylcellulose suspension	GLP Compliance:	Yes
Basis for High-Dose Selection:	Toxicity-based endpoint	Treatment of Controls:	0.5 w/v% methylcellulose solution		
Special Features:	Hematology evaluations at scheduled sacrifice. Toxicokinetic monitoring on satellite animals at starting day, Week 13 and Week 52; microscopic examinations of livers from toxicokinetic satellite animals euthanized after the Week 52 blood collections. Due to high incidence of mortality, the highest dose group (1000 mg/kg/day) was necropsied during Week 95.				

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M	F	M	F	M	F	M	F	M	F
Toxicokinetics – Week 52 (N: 3)										
TAK-375 C _{max} (µg/mL)	NA	NA	2.015	1.444	10.052	6.185	13.327	11.848	12.558	14.374
M-I C _{max} (µg/mL)	NA	NA	1.481	3.627	3.636	8.445	5.816	17.060	7.811	16.839
M-II C _{max} (µg/mL)	NA	NA	0.936	0.493	3.343	1.608	4.485	1.928	3.425	3.769
M-III C _{max} (µg/mL)	NA	NA	9.313	6.125	16.873	15.990	20.880	20.545	15.041	22.014
M-IV C _{max} (µg/mL)	NA	NA	1.831	0.827	3.960	1.867	2.995	1.852	2.891	2.563
TAK-375 AUC (µg·hr/mL)	NA	NA	0.967	0.722	10.138	7.773	50.425	21.061	55.003	48.829
M-I AUC (µg·hr/mL)	NA	NA	1.069	2.193	5.085	9.203	19.623	34.979	35.953	62.598
M-II AUC (µg·hr/mL)	NA	NA	0.614	0.285	7.203	2.789	37.761	7.996	28.427	14.496
M-III AUC (µg·hr/mL)	NA	NA	5.117	4.173	31.902	29.729	109.549	80.833	89.069	119.109
M-IV AUC (µg·hr/mL)	NA	NA	3.669	1.319	12.544	5.984	33.352	16.921	23.642	22.386
Number of Animals										
At Start	55	55	55	55	55	55	55	55	55	55
Died/Sacrificed Moribund	15	13	11	16	14	21	14	18	30	31
Terminal Sacrifice	40	42	44	39	41	34	41	37	25 (a)	24 (a)
Survival (%)	72.7	76.4	80.0	70.9	74.5	61.8	74.5	67.3	45.5&	43.6&
Noteworthy Findings										
Body Weight (%) (b)	33.5g	28.7g	+0.3	+1.7	0	+2.1	-1.8	+0.3	-5.7&	-0.7
Food Consumption (%) (b)	4.6g/day	4.5g/day	-2.2	+2.2	0	+4.4	-2.2	+2.2	-2.2	+6.7&

2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice
(continued)

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
Number Evaluated	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55
Noteworthy Findings										
Clinical Signs										
Decreased spontaneous movement (c)	0	0	-	-	-	-	-	-	46	20
Prone/lateral position (c)	0	0	-	-	-	-	-	-	22	16
Oligopnea (c)	0	0	-	-	-	-	-	-	34	22
Hematology (No. of Animals)	40	42	44	39	41	34	41	37	25	24
RBC ($\times 10^4/\mu\text{L}$)	-	777	-	772	-	813	-	790	-	883&
Hb (g/dL)	13.6	12.9	13.5	12.7	14.6	13.5	13.2	13.2	15.2&	15.5&
Ht (%)	41	39	41	39	44	41	39	40	43&	46&
MCV (fL)	-	51.1	-	50.2	-	50.3	-	51.3	-	52.1&
MCH (pg)	16.7	16.8	16.5	16.5	16.8&	16.6	16.9&	16.8	18.0&	17.6&
MCHC (%)	32.9	32.8	32.7	33.0	33.3	33.1	33.8&	32.8	35.1&	33.7&
Reticulocyte (%)	3.2	-	2.6	-	2.2	-	3.0	-	4.8&	-
Platelet ($\times 10^4/\mu\text{L}$)	153.7	114.4	148.0	109.7	162.0	114.8	187.1&	131.8&	225.6&	165.8&
Number of Animals with Neoplastic Lesions										
Liver										
Adenoma, hepatocellular	20\$	10\$	24	14	32*#	14	50*#	52*#	54*#	55*#
Carcinoma, hepatocellular	9\$	4\$	6	3	18*#	3	26*#	11*#	50*#	51*#
Hepatoblastoma	0\$	0\$	0	0	1	0	3#	1	10*#	3#
Hemangiosarcoma	0	3	4#	3	4#	1	0	0	0	0
Hemangioma	2	0	4	1	1	1	0	1	0	0
Ito cell tumor	0	1	0	0	0	1	0	0	0	0
Harderian gland										
Adenoma	6	3\$	15*#	3	14*#	10*#	16*#	10*#	11	9#
Adenocarcinoma	1	1	1	2	1	0	2	0	1	0

APPENDIX 4 (cont.)

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2.6.7.10A Carcinogenicity: Twenty-Four-Month Carcinogenicity Study of TAK-375 in Mice

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2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice
(continued)

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55
Number of Animals with Neoplastic										
Adrenal gland										
Adenoma, cortical cell	0	0	0	0	1	0	0	0	0	0
Pheochromocytoma	0	0	0	0	0	0	0	2	0	0
Pheochromocytoma, malignant	0	0	0	0	0	0	0	1	0	0
Aorta										
Hemangiosarcoma	0	0	0	0	0	0	1	0	0	0
Cecum										
Leiomyoma	0	0	0	1	0	0	0	0	0	0
Duodenum										
Papilloma	0	0	0	0	0	0	1	0	0	0
Polyp	0	0	1	0	0	0	0	0	0	0
Ileum										
Hemangioma	0	0	0	0	0	0	0	0	1	0
Femur										
Hemangioma	0	0	2	2	0	0	0	0	0	0
Hemangiosarcoma	0	0	0	1	1	0	0	1	0	0
Hemolymphoreticular										
Lymphoma, malignant	4	13	5	17	5	19	6	21	2	4*
Mastocytoma, malignant	0	0	1	0	0	0	0	0	0	0
Sarcoma, histiocytic	4	5	4	9	5	3	1	5	3	6
Heart										
Hemangiosarcoma	0	1	0	0	0	0	0	0	0	0
Kidney										
Adenoma, renal cell	0	0	0	0	0	0	1	0	0	0
Carcinoma, renal cell	0	0	0	0	0	0	1	0	0	0
Hemangiosarcoma	0	0	0	0	0	0	1	0	0	0

2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice
(continued)

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55
Number of Animals with Neoplastic Lesions										
Lung										
Adenoma, bronchiolo-aveolar	7\$	2	6	4	10	5	15*#	2	8#	3
Adenocarcinoma	5	2	2	0	1	5	0*	2	0*	2
Adenoma + adenocarcinoma	12	4	8	4	11	10#	15	4	8	5
Lymph node (mesenteric)										
Hemangioma	0	0	0	0	0	1	0	0	0	0
Hemangiosarcoma	0	1	0	0	0	0	0	0	0	0
Mammary gland										
Adenoma	NA	1	NA	0	NA	0	NA	0	NA	0
Ovary										
Cystadenoma	NA	0	NA	1	NA	1	NA	1	NA	0
Granulosa cell tumor, malignant	NA	0	NA	1	NA	0	NA	0	NA	0
Pituitary gland										
Adenoma, anterior	0	3	0	3	0	5	0	2	0	0
Adenoma, intermediated	0	1	0	0	1	0	2	0	0	0
Carcinoma, anterior	0	0	0	0	0	0	0	1	0	0
Carcinoma, intermediated	0	0	0	0	0	0	0	0	0	1
Skin										
Sarcoma, spindle cell	0	0	0	1	0	4#	0	1	0	1
Carcinoma, squamous cell	0	0	0	1	0	0	0	0	0	0
Osteosarcoma	0	0	0	0	0	1	0	0	0	0
Hemangiosarcoma	2	0	0	0	0	0	0	0	0	0
Spleen										
Hemangioma	2	2	0	1	2	0	1	1	1	0
Hemangiosarcoma	0	2	4#	4	0	1	0	0	0	0

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2.6.7.10A Carcinogenicity: Twenty-Four-Month Carcinogenicity Study of TAK-375 in Mice

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2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice
(continued)

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55
Number of Animals with Neoplastic Lesions										
Stomach										
Hemangioma	0	0	0	1	0	0	0	0	0	0
Papilloma	0	1	0	1	0	0	0	2	0	1
Polyp, adenomatous	0	1	0	0	0	0	0	0	0	1
Sternum										
Hemangioma	0	0	1	0	0	0	0	0	0	0
Thoracic cavity										
Hemangiosarcoma	0	1	0	0	0	0	0	0	0	0
Testis										
Leydig cell tumor	1	NA	1	NA	1	NA	0	NA	0	NA
Thyroid gland										
Adenoma, follicular cell	0	0	1	1	0	0	2	0	0	0
Carcinoma, follicular cell	0	0	0	0	0	0	0	0	0	1
Tongue										
Carcinoma, squamous cell	1	0	0	0	1	0	0	0	0	0
Urinary bladder										
Hemangiosarcoma	0	0	0	0	1	0	0	0	0	0
Uterus										
Polyp, endometrial stromal	NA	1	NA	3	NA	0	NA	0	NA	0
Hemangiosarcoma	NA	0	NA	0	NA	0	NA	1	NA	0
Vagina										
Polyp, vaginal stromal	NA	0	NA	1	NA	0	NA	0	NA	0
Vertebra										
Osteosarcoma	0	0	0	0	0	0	0	1	0	0
Zymbal gland										
Carcinoma, squamous cell	0	0	0	0	0	0	0	1	0	0

2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice
(continued)

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55
Gross Pathology										
Harderian gland										
Node	6	4	15	6	15	8	20	9	13	11
Liver										
Node	23	18	29	14	41	13	53	49	54	55
Uterus										
Endometrial cyst	NA	37	NA	33	NA	37	NA	32	NA	8
Stomach										
Focus, raised	2	1	3	2	1	0	4	2	25	27
Focus, discolored	2	1	4	5	5	3	6	3	9	11
Histopathology - Non Neoplastic Lesions										
Adrenal gland										
Hypertrophy, cortical cell, diffuse										
Slight	1	6	7	10	3	11	10	14	42	51
Mild	0	6	6	10	3	11	7	13	34	30
Moderate	1	0	1	0	0	0	1	1	6	13
Moderate	0	0	0	0	0	0	2	0	2	8
Epididymis										
Cell infiltration, lymphocytic										
Slight	6	NA	10	NA	6	NA	4	NA	21	NA
Mild	6		10		6		4		20	
Mild	0		0		0		0		1	
Kidney										
Pigmentation, tubular cell										
Slight	0	0	1	0	2	1	0	2	17	22
Mild	0	0	0	0	2	1	0	1	15	21
Moderate	0	0	0	0	0	0	0	1	2	0
Moderate	0	0	1	0	0	0	0	0	0	1

APPENDIX 4 (cont.)

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 2.6.7.10A Carcinogenicity: Twenty-Four-Month Carcinogenicity Study of TAK-375 in Mice

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2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice (continued)

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55
Histopathology - Non Neoplastic Lesions										
Glomerulosclerosis	5	6	5	2	4	0	6	3	23	5
Slight	4	6	5	2	4	0	6	3	17	4
Mild	1	0	0	0	0	0	0	0	5	1
Moderate	0	0	0	0	0	0	0	0	1	0
Liver										
Necrosis, single cell	2	1	0	1	4	1	2	0	17	2
Slight	2	0	0	1	4	1	1	0	16	2
Mild	0	0	0	0	0	0	1	0	1	0
Moderate	0	1	0	0	0	0	0	0	0	0
Necrosis, focal	2	0	4	0	7	0	4	6	3	4
Slight	1	0	1	0	4	0	1	6	2	1
Mild	1	0	0	0	3	0	1	0	1	2
Moderate	0	0	3	0	0	0	2	0	0	1
Pigmentation, sinusoidal lining cell	0	0	0	1	2	1	2	0	44	31
Slight	0	0	0	1	1	1	2	0	44	31
Mild	0	0	0	0	1	0	0	0	0	0
Hypertrophy, hepatocyte, central	0	0	3	1	3	1	27	1	5	0
Slight	0	0	3	1	3	1	14	1	1	0
Mild	0	0	0	0	0	0	10	0	1	0
Moderate	0	0	0	0	0	0	3	0	3	0
Hypertrophy, hepatocyte, periportal	0	0	0	1	0	0	3	0	15	16
Slight	0	0	0	1	0	0	2	0	9	14
Mild	0	0	0	0	0	0	0	0	3	2
Moderate	0	0	0	0	0	0	1	0	3	0

**2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice
(continued)**

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55
Histopathology - Non Neoplastic Lesions										
Altered cell focus, eosinophilic	6	3	20	1	19	9	33	31	27	25
Altered cell focus, basophilic	2	1	3	1	4	0	7	1	2	0
Altered cell focus, clear	1	0	3	0	2	0	1	1	1	1
Ovary										
Atrophy	NA	18	NA	12	NA	18	NA	23	NA	55
Slight		1		3		5		0		2
Mild		0		2		3		2		0
Moderate		8		1		4		4		3
Severe		9		6		6		17		50
Spleen										
Hematopoiesis, increased	18	24	17	36	22	24	22	34	46	48
Slight	6	9	4	16	7	8	9	12	9	9
Mild	7	9	7	13	8	13	7	12	26	27
Moderate	5	6	6	7	7	3	6	9	11	10
Severe	0	0	0	0	0	0	0	1	0	2
Stomach										
Erosion, glandular stomach	2	1	4	4	4	3	3	3	5	7
Slight	1	0	3	3	4	2	2	3	4	5
Mild	1	1	1	1	0	1	1	0	1	2
Hyperplasia, squamous, focal, forestomach	3	1	3	1	1	1	3	1	30	29
Cell infiltration, inflammatory, forestomach	1	0	1	1	1	0	1	0	24	16
Slight	1	0	1	0	1	0	1	0	17	12
Mild	0	0	0	0	0	0	0	0	7	4
Moderate	0	0	0	1	0	0	0	0	0	0

APPENDIX 4 (cont.)

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 2.6.7.10A Carcinogenicity: Twenty-Four-Month Carcinogenicity Study of TAK-375 in Mice

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2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice
 (continued)

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55
Histopathology - Non Neoplastic Lesions										
Seminal vesicle										
Atrophy	1	NA	4	NA	9	NA	4	NA	34	NA
Slight	1		4		9		3		32	
Mild	0		0		0		1		0	
Moderate	0		0		0		0		1	
Severe	0		0		0		0		1	
Thyroid gland										
Pigmentation, follicular cell	0	0	0	1	0	3	28	31	49	43
Slight	0	0	0	1	0	3	27	30	24	20
Mild	0	0	0	0	0	0	1	1	25	23
Hypertrophy, follicular cell	0	0	0	0	1	1	2	5	33	46
Slight	0	0	0	0	1	1	2	5	32	46
Mild	0	0	0	0	0	0	0	0	1	0
Uterus										
Atrophy	NA	3	NA	10	NA	8	NA	13	NA	54
Slight		0		4		1		1		1
Mild		0		1		3		6		1
Moderate		2		3		2		4		4
Severe		1		2		2		2		48
Hyperplasia, endometrial, cystic	NA	53	NA	47	NA	50	NA	49	NA	28
Slight		14		16		17		21		27
Mild		30		25		29		24		1
Moderate		9		6		4		4		0

2.6.7.10A Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Mice
(continued)

Daily Dose (mg/kg)	0 (Control)		30		100		300		1000	
	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55	M: 55	F: 55
Histopathology - Non Neoplastic Lesions										
Vagina										
Atrophy	NA	4	NA	11	NA	8	NA	16	NA	50
Slight		0		3		0		1		1
Mild		0		2		3		2		2
Moderate		1		2		1		6		3
Severe		3		4		4		7		44
Pathology - Toxicokinetic Animals (d)										
Number of Animals Examined	0	0	21	21	21	21	21	21	22	21
Gross Pathologic Findings										
Liver										
Nodule	NA	NA	3	0	2	0	5	0	17	16
Histopathologic Findings										
Liver										
Adenoma, hepatocellular	NA	NA	1	0	1	0	1	0	6	6
Altered cell focus, eosinophilic	NA	NA	0	0	1	0	2	0	6	6

NA indicates not applicable; -, No treatment-related effects.

(a) Animals were necropsied during Week 95.

(b) At 6 months. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not on the percent differences).

(c) Week 1 only.

(d) Gross and histopathologic examinations were conducted on the liver from toxicokinetic satellite animals at Week 52.

& $p \leq 0.05$ (significantly different from the control group).

* $p \leq 0.05$ (significantly different from the control group, Fisher's exact test).

S $p \leq 0.05$ (significantly different from the control group in pooled [fatal + incidental] tumors, in Petos positive trend test of all groups).

$p \leq 0.05$ (significantly different from the control group in pooled [fatal + incidental] tumors, in pairwise comparison between each dose group and the control group in Petos positive trend test).

APPENDIX 4 (cont.)

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2.6.7.10B Carcinogenicity: Twenty-Four-Month Carcinogenicity Study of TAK-375 in Rats

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2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats

Species/Strain:	CD(SD)IGS Rats	Duration of Dosing:	24 Months	Test Article:	TAK-375
Initial Age:	6 Weeks	Method of Administration:	Gavage	Study/Report No:	B-4416/M-11-00561.002A
Date of First Dose:	30 Nov 99 (males)	Vehicle/Formulation:	0.5 w/v% methylcellulose suspension	GLP Compliance:	Yes
	1 Dec 99 (females)	Treatment of Controls:	0.5 w/v% methylcellulose solution		

Basis for High-Dose Selection: Maximum tolerated dose was not found in the 26-week and 4-week oral gavage toxicity studies up to 600 mg/kg/day. In a 2-week oral gavage preliminary toxicity study, 1 male died and 3 females were sacrificed in extremis at 2000 mg/kg/day.

Special Features: Hematology evaluations at scheduled sacrifice. Toxicokinetic monitoring conducted on 10 satellite males and females per treated group bled at starting day, Week 26 and Week 52; microscopic examinations of liver and testis from toxicokinetic animals euthanized after the Week 52 blood collections. Due to high incidence of mortality, the highest dose females were necropsied during Week 94.

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M	F	M	F	M	F	M	F	M	F
Toxicokinetics – Week 52 (N: 3)										
TAK-375 C _{max} (µg/mL)	NA	NA	3.053	3.610	7.822	16.452	12.175	23.059	22.333	52.831
M-I C _{max} (µg/mL)	NA	NA	1.955	1.588	5.301	3.573	7.295	4.492	9.404	4.385
M-II C _{max} (µg/mL)	NA	NA	0.427	1.250	0.892	3.048	0.804	3.671	1.206	4.088
M-III C _{max} (µg/mL)	NA	NA	2.414	2.615	5.780	8.530	9.055	14.016	12.292	21.194
M-IV C _{max} (µg/mL)	NA	NA	0.320	0.592	0.356	0.741	0.274	0.970	0.482	1.036
TAK-375 AUC (µg·hr/mL)	NA	NA	4.569	4.434	13.432	26.478	42.033	91.839	171.041	230.770
M-I AUC (µg·hr/mL)	NA	NA	4.332	2.462	9.438	6.072	21.755	13.434	59.586	27.073
M-II AUC (µg·hr/mL)	NA	NA	2.074	3.773	2.953	9.945	3.359	22.278	12.194	35.379
M-III AUC (µg·hr/mL)	NA	NA	7.412	7.554	14.189	25.170	38.089	98.970	153.732	164.879
M-IV AUC (µg·hr/mL)	NA	NA	1.968	3.685	1.565	3.981	1.658	9.208	6.178	13.410

**2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)**

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Number Evaluated										
Number of Animals										
At Start	120 (a)	120 (a)	60	60	60	60	60	60	60	60
Died/Sacrificed Moribund	84	75	37	43	34	35	26	32	35	36
Terminal Sacrifice	36	45	23	17	26	25	34	28	25	24
Survival (%)	30.0	37.5	38.3	28.3	43.3	41.7	56.7	46.7	41.7	40.0 (b)&
Noteworthy Findings										
Body Weight (%) (c)	671g	338g	-3	-6&	-6&	-2&	-8&	-9&	-25&	-20&
Food Consumption (%) (c)	31g/day	22g/day	-3	-5	-3	0	-6&	-5	0	-5
Clinical Signs										
Decreased spontaneous movement	25	43	9	19	8	25	11	18	14	20
Abnormal gait	0	0	0	0	0	0	0	0	5	7
Convulsion/clonic	5	1	3	0	3	2	3	3	12	8
Oligopnea	31	41	11	20	14	23	13	13	29	31
Hematology (No. of Animals)	M: 36	F: 44	M: 23	F: 17	M: 26	F: 25	M: 34	F: 28	M: 25	F: 24
RBC (x10 ⁴ /μL)	643	-	676	-	715	-	656	-	732&	-
Hb (g/dL)	-	12.8	-	13.2	-	12.8	-	12.7	-	11.8&
Ht (%)	-	39	-	40	-	38	-	38	-	34&
MCV (fL)	57.5	60.7	56.9	59.7	56.3	59.8	57.2	57.5&	51.9&	53.6&
MCH (pg)	19.1	20.2	19.3	19.8	19.1	20.1	19.2	19.2&	18.2&	18.6&
MCHC (%)	33.2	33.3	34.0	33.3	33.9	33.6	33.6	33.4	35.1&	34.7&
Reticulocyte (%)	-	3.5	-	2.2	-	3.2	-	2.0	-	3.1&
Platelet (x10 ⁴ /μL)	118.0	87.0	108.8	88.5	107.5	90.6	114.4	96.0	152.5&	138.6&
WBC (x10 ² /μL)	-	63	-	59	-	75	-	65	-	86&

APPENDIX 4 (cont.)

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2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Number Evaluated										
Number of Animals with Neoplastic Lesions										
Liver										
Adenoma, hepatocellular	3\$	2\$	7*#(f)	0	7*#(f)	8*#	15*#	22*#	47*#	49*#
Carcinoma, hepatocellular	0\$	0\$	0	0	0	1	0	1	38*#	20*#
Hepatoblastoma	0	0	0	0	0	0	0	0	1	0
Cholangioma	0	0	0	0	0	0	1	1	1	0
Hemangioma	0	0	0	0	0	0	0	1	0	1
Hemangiosarcoma	0	0	0	0	0	2	0	1	1	1
Testis										
Leydig cell tumor	3\$	NA	1	NA	0	NA	7*#(e)	NA	46*#	NA
Abdominal cavity										
Choriocarcinoma	0	0	0	0	0	1	0	0	0	0
Mesothelioma, malignant	1	0	0	0	0	0	0	0	0	0
Abdominal wall										
Lipoma	0	0	0	0	0	1	0	0	0	0
Adrenal gland										
Adenoma, cortical cell	2	4	1	1	2	1	0	4	2	2
Paraganglioma	0	0	0	0	0	0	0	1	0	0
Pheochromocytoma	11	6	9	0	6	3	2	1	1*	1
Pheochromocytoma, malignant	0	2	1	0	0	1	1	0	0	0
Carcinoma, cortical cell	0	1	0	1	0	0	0	0	0	0
Cerebellum										
Granular cell tumor	0	0	0	0	0	0	1	0	0	1
Meningioma	1	0	0	0	0	0	0	0	0	0
Astrocytoma, malignant	0	0	1	0	0	0	0	0	0	0

APPENDIX 4 (cont.)

2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Number of Animals with Neoplastic Lesions										
Kidney										
Carcinoma, renal cell	0	0	0	0	1	0	0	0	0	0
Nephroblastoma	0	0	0	0	0	1	0	0	0	0
Schwannoma, malignant	1	0	0	0	0	0	0	0	0	0
Limb										
Osteosarcoma	0	0	0	0	0	0	1	0	0	0
Lung										
Adenoma, bronchiolo-alveolar	0	0	0	0	0	0	1	0	1	0
Epithelioma, cystic, keratinizing	0	0	0	0	0	0	0	0	0	1
Adenocarcinoma	0	0	0	0	0	0	0	1	0	0
Carcinoma, squamous cell	1	0	0	0	0	0	0	0	0	0
Lymph node, mesenteric										
Hemangioma	3	0	0	0	1	0	0	0	0	0
Lymphangioma	1	0	0	0	0	0	0	0	0	0
Mammary gland										
Adenoma	1	2	0	4 #	0	3	0	3	0	0
Fibroadenoma	1	56	2	21	0	25	0	20	0	4*
Adenocarcinoma	0	29	0	14	0	17	0	11	0	0*
Hemangiosarcoma	0	1	0	0	0	0	0	0	0	0
Oral cavity										
Carcinoma, squamous cell	1	0	0	0	0	0	0	0	0	1
Ovary										
Granulosa cell tumor	NA	0	NA	0	NA	0	NA	1	NA	0
Adenoma, sertoliform	NA	0	NA	0	NA	0	NA	1	NA	0

2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Number of Animals with Neoplastic Lesions										
Pancreas										
Adenoma, acinar cell	6	2	7	0	7	2	7	3	2	0
Adenoma, islet cell	47	10	16	8	23	6	28	4	15*	0*
Carcinoma, islet cell	3	1	0	0	0	0	1	0	0	0
Hemangiosarcoma	0	0	0	0	0	0	0	0	0	1
Parathyroid gland										
Adenoma	0	1	0	0	0	0	1	0	2	0
Pituitary gland										
Adenoma, anterior	84	97	42	48	42	44	40	45	22*	22*
Adenoma, intermediate	0	0	0	0	0	0	0	0	1	0
Carcinoma	0	5	0	4	1	1	1	3	0	0
Prostate										
Adenocarcinoma	1	NA	1	NA	0	NA	0	NA	0	NA
Rectum										
Granular cell tumor	0	0	0	0	0	0	0	0	0	1
Carcinoma, squamous cell	0	0	0	0	0	0	1	0	0	0
Salivary gland										
Parotid: carcinoma, acinar cell	0	0	0	0	0	0	0	1	0	0
Skin										
Adenoma, sebaceous cell	1	1	0	0	1	0	0	0	0	0
Fibroma	7	0	3	0	3	0	2	0	1	0
Keratoacanthoma	1	1	4**	0	2	1	4**	0	0	0
Lipoma	2	2	1	1	0	0	0	0	0	0

APPENDIX 4 (cont.)

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2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
Number Evaluated	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Number of Animals with Neoplastic Lesions										
Skin										
Melanoma, benign, amelanotic	0	0	0	0	0	0	0	0	1	0
Papilloma, squamous cell	2	0	5*#	0	2	0	4 #	0	1	0
Trichoepithelioma	0	0	0	0	0	1	0	0	0	0
Tumor, basal cell, malignant	0	0	1	0	1	0	0	0	0	0
Carcinoma, squamous cell	1	0	0	0	0	0	0	0	0	0
Fibrosarcoma	4	1	2	0	0	0	2	0	1	0
Hemangiopericytoma, malignant	1	0	0	0	0	0	0	0	0	0
Melanoma, amelanotic	0	0	0	0	0	0	1	0	0	0
Osteosarcoma	1	0	0	0	0	0	0	0	0	0
Rhabdomyosarcoma	0	0	1	0	0	0	0	0	0	0
Sarcoma, nos	0	2	0	1	0	0	0	0	0	0
Spinal cord										
Granular cell tumor	0	0	0	0	1	0	0	1	0	0
Astrocytoma, malignant	1	0	0	0	0	0	0	0	0	0
Spleen										
Sarcoma, nos	0	0	0	0	1	0	0	0	0	0
Stomach										
Leiomyoma	0	0	0	0	0	0	0	0	1	0
Carcinoma, squamous cell	1	0	0	1	0	0	0	0	1	0
Leiomyosarcoma	0	0	0	0	0	0	0	0	1	0
Thyroid gland										
Adenoma, C cell	8	5	2	7 #	4	5	2	1	3	0
Adenoma, follicular cell	1	0	2	0	0	1	4 #	0	1	0
Carcinoma, C cell	1	3	0	0	0	2	1	1	1	0
Carcinoma, follicular cell	0	0	0	1	1	0	0	0	1	0

**2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)**

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Number Evaluated										
Number of Animals with Neoplastic Lesions										
Urinary bladder										
Lipoma	0	0	0	0	1	0	0	0	0	0
Papilloma, transitional cell	0	1	0	0	1	1	0	1	0	0
Carcinoma, transitional cell	0	0	0	0	0	0	0	0	2	0
Uterus										
Adenoma	NA	0	NA	0	NA	0	NA	1	NA	0
Granular cell tumor	NA	3	NA	1	NA	1	NA	3	NA	1
Hemangioma	NA	0	NA	1	NA	0	NA	0	NA	0
Polyp, adenomatous	NA	2	NA	0	NA	0	NA	0	NA	0
Polyp, endometrial stromal	NA	8	NA	5	NA	2	NA	7	NA	2
Adenocarcinoma	NA	0	NA	0	NA	0	NA	0	NA	1
Carcinoma, squamous cell	NA	0	NA	0	NA	0	NA	1	NA	0
Schwannoma, malignant	NA	0	NA	0	NA	0	NA	1	NA	0
Vagina										
Granular cell tumor	NA	6	NA	2	NA	1	NA	5	NA	0
Leiomyosarcoma	NA	1	NA	0	NA	0	NA	0	NA	0
Schwannoma, malignant	NA	0	NA	1	NA	1	NA	0	NA	0
Zymbal gland										
Carcinoma	0	0	1	0	0	0	0	0	0	0
Gross Pathology										
Adrenal gland										
Discoloration, yellowish white	0	0	0	0	0	0	0	0	7	0
Large	9	21	6	7	2	13	5	20	15	11

APPENDIX 4 (cont.)

Ramelteon
 2.6.7.10B Carcinogenicity: Twenty-Four-Month Carcinogenicity Study of TAK-375 in Rats

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2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
 (continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Gross Pathology										
Extraorbital lacrimal gland										
Discoloration, dark	0	0	0	0	0	0	0	12	4	32
Kidney										
Discoloration, dark	0	0	1	0	0	0	0	0	6	2
Liver										
Discoloration, dark	0	0	0	0	0	0	1	12	42	49
Large	0	1	1	1	0	1	6	12	50	53
Node	3	4	6	0	2	7	10	19	46	49
Focus, discolored	21	27	11	17	20	22	29	33	46	36
Lung (bronchus)										
Focus, discolored	4	3	5	3	4	5	8	5	17	13
Prostate										
Small	2	NA	1	NA	2	NA	3	NA	10	NA
Seminal vesicle										
Small	4	NA	1	NA	2	NA	4	NA	17	NA
Testis										
Focus, discolored	2	NA	1	NA	0	NA	7	NA	46	NA
Thyroid gland										
Discoloration, dark	0	0	0	0	0	0	26	9	53	53
Histopathology - Non Neoplastic Lesions										
Adrenal gland										
Vacuolation, cortical cell, increased	17	3	9	1	18	28	41	48	52	47
Slight	14	2	9	1	11	26	14	30	3	26
Mild	3	1	0	0	7	2	22	18	25	17
Moderate	0	0	0	0	0	0	5	0	24	4

2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Histopathology - Non Neoplastic Lesions										
Vacuolation, cortical cell, focal	21	7	7	4	6	3	16	3	46	14
Hypertrophy, cortical cell, diffuse	1	5	1	3	9	9	35	48	34	46
Slight	1	5	0	3	9	9	25	29	13	12
Mild	0	0	1	0	0	0	9	19	18	26
Moderate	0	0	0	0	0	0	1	0	3	8
Cerebellum										
Pigmentation, PAS-negative, increased	0	0	0	0	0	0	10	10	50	45
Slight	0	0	0	0	0	0	10	10	12	39
Mild	0	0	0	0	0	0	0	0	38	6
Cerebrum										
Pigmentation, PAS-negative, increased	0	0	0	0	0	0	11	8	50	46
Slight	0	0	0	0	0	0	11	8	50	46
Mineralization										
Slight	2	2	0	0	1	0	2	0	28	6
Mild	0	0	0	0	0	0	0	0	7	1
Moderate	0	0	0	0	0	0	0	0	3	0
Epididymis										
Atrophy	34	NA	21	NA	8	NA	9	NA	28	NA
Slight	12		10		3		5		5	
Mild	14		7		2		3		13	
Moderate	8		4		3		1		10	
Hypospermia	30	NA	18	NA	8	NA	8	NA	36	NA
Slight	7		2		1		0		1	
Mild	9		7		1		0		2	
Moderate	4		4		1		4		7	
Severe	10		5		5		4		26	

APPENDIX 4 (cont.)

Ramelteon
 2.6.7.10B Carcinogenicity: Twenty-Four-Month Carcinogenicity Study of TAK-375 in Rats

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2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
 (continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Histopathology – Non Neoplastic Lesions										
Extraorbital lacrimal gland										
Pigmentation, acinar cell, increased										
Slight	1	5	1	2	0	5	1	23	21	44
Mild	1	4	1	2	0	4	1	23	19	42
Moderate	0	1	0	0	0	1	0	0	2	2
Eye										
Atrophy, retinal	12	34	10	12	10	17	8	12	21	14
Slight	12	25	6	8	6	15	5	9	11	9
Mild	0	8	2	4	2	2	1	3	8	2
Moderate	0	1	2	0	2	0	2	0	2	3
Femur (with marrow)										
Atrophy, trabecular bone	40	6	11	4	19	5	26	9	43	17
Slight	6	2	3	3	5	4	6	8	1	8
Mild	19	4	5	1	13	1	11	1	10	9
Moderate	15	0	3	0	1	0	9	0	32	0
Kidney										
Chronic progressive nephropathy	77	28	33	11	44	19	49	23	44	40
Slight	35	14	14	4	11	4	13	13	11	11
Mild	27	10	9	6	23	11	26	7	26	14
Moderate	9	2	4	1	9	4	7	2	6	12
Severe	6	2	6	0	1	0	3	1	1	3
Kidney										
Increased pigmentation, tubular cell	5	17	1	6	0	13	6	29	39	54
Slight	4	17	1	6	0	12	5	26	38	15
Mild	0	0	0	0	0	0	1	3	1	39
Moderate	1	0	0	0	0	1	0	0	0	0

2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Histopathology - Non Neoplastic Lesions										
Liver										
Necrosis, focal	17	9	6	6	9	5	9	9	37	35
Slight	12	5	4	4	5	4	7	4	8	11
Mild	5	4	2	2	4	1	1	5	27	22
Moderate	0	0	0	0	0	0	1	0	2	2
Pigmentation, sinusoidal lining cell	0	0	0	0	0	0	0	3	54	50
Slight	0	0	0	0	0	0	0	3	53	42
Mild	0	0	0	0	0	0	0	0	1	8
Pigmentation, hepatocytic	0	0	0	0	0	0	2	51	47	55
Slight	0	0	0	0	0	0	2	41	25	4
Mild	0	0	0	0	0	0	0	10	16	21
Moderate	0	0	0	0	0	0	0	0	6	30
Hemorrhage	2	3	2	0	1	1	0	2	19	13
Slight	1	1	2	0	1	0	0	1	6	3
Mild	1	2	0	0	0	0	0	1	13	10
Moderate	0	0	0	0	0	1	0	0	0	0
Fibrosis	7	5	2	4	5	9	6	5	43	46
Slight	5	4	2	4	4	8	6	4	15	7
Mild	2	1	0	0	1	1	0	1	28	39
Hypertrophy, hepatocellular, central	0	0	3	0	37	14	49	48	50	36
Slight	0	0	3	0	31	13	38	23	9	12
Mild	0	0	0	0	6	1	8	12	11	9
Moderate	0	0	0	0	0	0	3	11	29	15
Severe	0	0	0	0	0	0	0	2	1	0

2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Histopathology - Non Neoplastic Lesions										
Liver										
Altered cell focus, clear	37	32	20	20	32	26	33	30	39	25
Slight	33	32	14	19	24	22	26	22	17	9
Mild	4	0	5	1	6	4	7	7	15	6
Moderate	0	0	1	0	1	0	0	1	7	7
Severe	0	0	0	0	1	0	0	0	0	3
Altered cell focus, eosinophilic	33	36	27	25	43	38	52	50	51	45
Slight	28	32	23	25	22	31	26	35	5	18
Mild	4	4	3	0	17	6	19	10	9	17
Moderate	1	0	1	0	4	1	6	4	25	6
Severe	0	0	0	0	0	0	1	1	12	4
Altered cell focus, mixed	0	4	0	2	3	9	10	28	14	20
Slight	0	4	0	2	3	9	9	25	11	11
Mild	0	0	0	0	0	0	1	3	3	8
Moderate	0	0	0	0	0	0	0	0	0	1
Altered cell focus, tigroid	51	90	33	46	33	50	28	45	20	14
Slight	48	77	31	39	33	42	28	33	20	13
Mild	3	12	2	7	0	7	0	11	0	1
Moderate	0	1	0	0	0	1	0	1	0	0
Lung										
Hemorrhage	2	7	3	2	6	8	9	13	12	12
Slight	2	6	2	2	5	6	7	10	9	11
Mild	0	1	1	0	1	2	2	3	3	1
Inflammation, interstitial, focal	4	3	3	4	4	3	17	10	35	26
Slight	3	3	3	4	3	2	13	9	29	22
Mild	1	0	0	0	1	1	4	1	6	4

APPENDIX 4 (cont.)

2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
Number Evaluated	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Histopathology - Non Neoplastic Lesions										
Histiocytosis, alveolar	55	60	28	30	42	41	52	52	49	50
Slight	30	32	10	17	26	20	19	19	29	25
Mild	24	23	18	12	14	19	30	28	19	22
Moderate	1	5	0	1	2	2	3	5	1	3
Histiocytosis, alveolar, pigmented	2	1	0	0	5	3	7	5	32	25
Slight	1	1	0	0	5	2	4	3	21	21
Mild	1	0	0	0	0	1	3	2	10	4
Moderate	0	0	0	0	0	0	0	0	1	0
Prostate										
Atrophy	100	NA	56	NA	54	NA	59	NA	55	NA
Slight	17		8		8		5		7	
Mild	63		33		38		43		15	
Moderate	20		14		7		11		32	
Severe	0		1		1		0		1	
Salivary gland, submandibular										
Pigmentation, macrophage	0	0	0	0	0	0	0	0	9	9
Slight	0	0	0	0	0	0	0	0	9	9
Salivary gland, sublingual										
Pigmentation, macrophage	0	0	0	0	0	0	0	0	3	4
Slight	0	0	0	0	0	0	0	0	3	4
Seminal vesicle										
Atrophy	34	NA	12	NA	8	NA	10	NA	33	NA
Slight	7		0		1		0		1	
Mild	12		1		2		4		5	
Moderate	15		11		5		6		27	

2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Histopathology - Non Neoplastic Lesions										
Spinal cord										
Pigmentation, PAS-negative, increased	0	0	0	0	0	0	8	12	49	45
Slight	0	0	0	0	0	0	8	12	8	36
Mild	0	0	0	0	0	0	0	0	41	9
Testis										
Atrophy, seminiferous tubular	31	NA	17	NA	11	NA	18	NA	43	NA
Slight	10		4		3		6		2	
Mild	8		3		3		3		4	
Moderate	0		2		0		1		3	
Severe	13		8		5		8		34	
Hyperplasia, Leydig cell, focal	7	NA	2	NA	9	NA	18	NA	48	NA
Thyroid gland										
Pigmentation, follicular cell	0	0	0	0	2	0	39	25	46	45
Slight	0	0	0	0	2	0	7	24	4	9
Mild	0	0	0	0	0	0	26	1	7	23
Moderate	0	0	0	0	0	0	6	0	35	13
Thyroid gland										
Small follicle, increased	6	3	1	2	5	7	24	13	45	37
Slight	6	3	1	2	5	6	18	12	39	23
Mild	0	0	0	0	0	1	6	1	6	14
Hyperplasia, follicular, focal	5	2	7	3	6	3	6	1	12	5

APPENDIX 4 (cont.)

2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
Number Evaluated	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Histopathology - Non Neoplastic Lesions										
Pathology - Toxicokinetic Animals (d)										
Number of Animals Examined	0	0	9	10	10	9	9	9	10	5
Gross Pathologic Findings										
Liver										
Discoloration, dark	NA	NA	0	0	0	0	0	0	10	5
Large	NA	NA	0	0	0	0	0	0	10	5
Node/nodule	NA	NA	0	0	0	0	1	0	0	4
Focus, discolored	NA	NA	0	0	0	0	0	2	7	5
Testis										
Focus, discolored	NA	NA	0	NA	0	NA	0	NA	3	NA
Animals with Neoplastic Lesions										
Liver										
Adenoma, hepatocellular	NA	NA	0	0	0	0	1	1	3	4
Testis										
Leydig cell tumor	NA	NA	0	NA	0	NA	0	NA	3	NA
Liver										
Necrosis, focal	NA	NA	0	0	0	0	0	0	1	1
Necrosis, single cell	NA	NA	0	0	0	0	0	0	1	0
Pigmentation, hepatocyte	NA	NA	0	0	0	0	0	8	10	5
Fibrosis										
Hypertrophy, hepatocellular, central	NA	NA	0	0	9	9	9	9	10	5
Proliferation, small bile duct	NA	NA	1	2	3	1	2	1	0	0
Spongiosis hepatis	NA	NA	0	0	0	0	1	0	0	0

2.6.7.10B Carcinogenicity: Report Title: Twenty-Four-Month Oral Gavage Carcinogenicity Study of TAK-375 in Rats
(continued)

Daily Dose (mg/kg)	0 (Control)		15		60		250		1000	
	M: 120	F: 120	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60	M: 60	F: 60
Pathology – Toxicokinetic Animals (d)										
Number of Animals Examined	0	0	9	10	10	9	9	9	10	5
Animals with Non-Neoplastic Lesions										
Altered cell focus, amphophilic	NA	NA	0	0	0	0	0	0	6	3
Altered cell focus, basophilic	NA	NA	1	1	0	1	2	2	6	3
Altered cell focus, clear	NA	NA	2	1	2	0	3	3	1	4
Altered cell focus, eosinophilic	NA	NA	2	1	0	1	4	5	7	5
Altered cell focus, tigroid	NA	NA	0	6	1	1	0	2	0	1
Testis										
Atrophy, seminiferous tubule	NA	NA	0	NA	0	NA	0	NA	1	NA
Hyperplasia, Leydig cell, diffuse	NA	NA	0	NA	0	NA	0	NA	8	NA
Hyperplasia, Leydig cell, focal	NA	NA	1	NA	2	NA	4	NA	10	NA

NA indicates not applicable; -, No treatment-related effects.

(a) Consists of two control groups each with 60 rats/sex/group (Control-I and Control-II).

(b) 1000-mg/kg/day females were necropsied during Week 94.

(c) At 6 months. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not on the percent differences).

(d) Gross and histopathologic examinations were conducted on the liver from satellite animals at Week 52.

(e) No significant difference from Control I in Fishers exact and Petos test.

(f) No significant difference from Control II in Fischers exact and Petos test.

& $p \leq 0.05$ (significantly different from the control group [Control I and Control II combined]).

* $p \leq 0.05$ (significantly different from the control group [Control I and Control II combined], Fishers exact test).

§ $p \leq 0.05$ (significantly different from the control group [Control I and Control II combined] in pooled [fatal + incidental] tumors, in Petos positive trend test of all groups).

$p \leq 0.05$ (significantly different from the control group [Control I and Control II combined] in pooled [fatal + incidental] tumors in pairwise comparison between each dose group and the combined control group, Petos positive trend test).

APPENDIX 4 (cont.)

Ramelteon

2.6.7.11 Reproductive and Developmental Toxicity: Nonpivotal Studies

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2.6.7.11 Reproductive and Developmental Toxicity: Nonpivotal Studies

Test Article: TAK-375

Species/Strain	Method of Admin. (Vehicle/Formulation)	Dosing Period	Dose mg/kg/day	No. per Group	Noteworthy Findings	Study/Report Number
\ CD(SD) IGS Rats	Gavage (0.5% MC)	G6 through G17	0, 10, 100, and 1000	6 pregnant females	Dams 100 mg/kg/day: ↑ post implantation loss. 1000 mg/kg/day: Mortality (4): ↓ fecal pellets; ↓ spontaneous activity; lying on side, bradypnea, lacrimation, mydriasis, salivation; ↓ BWC; ↓ FC; discolored liver, enlarged adrenal glands; small thymus, ↑ liver weight; ↑ adrenal gland weight; ↓ number of implantation sites; ↑ post implantation loss Fetuses 1000 mg/kg/day: ↓ live fetuses; ↓ fetal BW.	2630/te/ M-11-12.001
\ CD(SD) IGS Rats	Gavage (0.5% MC)	G6 through L6	0, 30, 100, and 300	7 Pregnant Females (control: 6)	Dams 300 mg/kg/day: ↓ BW; ↓ BWC (during gestation); ↓ FC; ↓ locomotor activity, salivation.	300319P/ M-11-00294
\ CD(SD) IGS Rats	IV (30% PEG)	G6 through G17	M-II (2S, 8S): 0, 10, 20, and 30	6 pregnant females	No noteworthy findings	3083/te/ M-11-168
\ JW Rabbits	Gavage (0.5% MC)	G6 through G18	0, 30, 100, 300, and 1000	3-6 Pregnant Females	Dams 100 mg/kg/day: creeping; myosis; ↓ spontaneous activity, ↓ FC. 300 mg/kg/day: lying on side, creeping, ↓ spontaneous activity, myosis, ↓ fecal pellets; ↓ FC. 1000 mg/kg/day: mortality (all); lying on side, creeping, convulsions, rigidity, bradypnea, dyspnea, myosis.	2698/te/ M-11-00612

- indicates No noteworthy findings. 0.5% MC, 0.5 w/v% methylcellulose solution; 30% PEG, 30% vol PEG 400 solution; ↑, increased, ↓, decreased; BW, body weight; BWC, body weight change; FC, food consumption; G, Gestation day, IV, intravenous injection; L, Lactation day.
M-II (2S, 8S) is a metabolite of TAK-375.

2.6.7.12A Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation Report
Title: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats

Design similar to ICH 4.1.1?:	Yes	Duration of Dosing:	M: 10 weeks (4 weeks pre-mating through day before necropsy) F: 2 weeks pre-mating through Gestation Day 6	Test Article:	TAK-375
Species/Strain:	\ CD(SD)IGS Rats	Day of Mating:	Day 0	Study/Report No:	2702/FE/M-11-37
Initial Age:	M: 11 weeks; F: 10 weeks	Day of C-Section:	Day 13 of gestation	GLP Compliance:	Yes
Special Features:	None	Vehicle/Formulation:	0.5 w/v% methylcellulose solution		
No Observed Adverse Effect Level:					
F0 Males:	60 mg/kg/day				
F0 Females:	6 mg/kg/day				
F1 Litters:	600 mg/kg/day				

Daily Dose (mg/kg)		0 (Control)	6	60	600
Treated Males	Toxicokinetics (a)				
	C _{max} (µg/mL)	NA	0.40	8.74	28.66
	AUC (µg-hr/mL)	NA	0.6	26.3	230.5
	No. Evaluated	20	20	20	20
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations				
	Creeping	-	-	-	2
	Crouching	-	-	-	7
	Lying on side	-	-	-	2
	Decrease in spontaneous activity	-	-	-	17
	Ataxic gait	-	-	-	6
	Salivation	-	-	1	20
	Chromaturia	-	-	-	20

APPENDIX 4 (cont.)

Ramelteon
 2.6.7.12A Reproductive and Developmental Toxicity: Effects of TAK-375 on Fertility
 and Early Embryonic Development to Implantation in Rats

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2.6.7.12A Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation Report
 Title: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats (continued)

Daily Dose (mg/kg)		0 (Control)	6	60	600
Treated Males	Necropsy Observations				
	Liver enlarged	0	0	0	12
	Liver - dark	0	0	0	11
	Body Weight (%) (b)	555 g	-0.9	-2.3	-8.3**
	Food Consumption (%) (c)	29 g	-3.4	-3.4	-31.0**
	Organ Weight (%) (d)				
	Liver (g)	18.17	-	-	+52.2**
	Adrenal glands (mg)	46.5	-	-	+48.2**
	Histopathology	-	-	-	-
	Sperm Examinations				
	No. Sperm (×106/g)	659	617	623	622
	Sperm Motility (%)	89.6	87.9	82.2	86.2
Sperm Abnormalities (%)	5.2	1.9	6.6	2.0	
Treated Males/Treated Females	Mean No. Days Prior to Mating	2.4	2.7	1.9	3.8
	No. of Males that Mated	20	20	20	11 (e)
	No. of Fertile Males	19	20	19	10 (e)
Treated Females	Toxicokinetics (a)				
	Cmax (µg/mL)	NA	1.24	15.13	78.50
	AUC (µg-hr/mL)	NA	1.3	67.8	1253.1
	No. Evaluated	20	20	20	20
	No. Died or Sacrificed Moribund	0	0	0	9
	Clinical Observations				
	Creeping	-	-	-	7
	Crouching	-	-	-	9
	Lying on side	-	-	-	11
	Decrease in spontaneous activity	-	-	-	9
Ataxic gait	-	-	-	2	

2.6.7.12A Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation Report
Title: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats (continued)

Daily Dose (mg/kg)		0 (Control)	6	60	600
Treated Females	Clinical Observations				
	Bradypnea	-	-	-	10
	Salivation	-	-	-	12
	Chromaturia	-	-	-	1
	Decrease in fecal pellets	-	-	-	10
	Necropsy Observations (f)				
	Petechiae in stomach	0	0	0	3
	Enlarged adrenal glands	0	0	0	7
	Discolored spleen	0	0	0	3
	Congestion in lung	0	0	0	6
	Premating Body Weight (%) (h)	257 g	+3.5	0	-2.3
	Gestation Body Weight (%) (h)	325 g	+1.2	-3.4	-6.2**
	Premating Food Consumption (%) (h)	19 g	+5.3	0	-26.3**
	Gestation Food Consumption (%) (i)	24 g	0	-8.3	-12.5*
	Organ Weight (%) (d)				
	Liver (g)	13.55	-1.2	-6.0	+18.1**
Adrenal glands (mg)	69.0	+4.9	-3.9	+18.3**	
Treated Females/Treated Males	No. Irregular Estrous Cycles (%)	0 (0.0)	0 (0.0)	10 (50.0**)	6 (54.6**)
	Mean No. Days Prior to Mating	2.4	2.7	1.9	3.8
	No. of Females Sperm Positive	20	20	20	11
	No. of Pregnant Females	19	20	19	10
	No. Aborted or with Total Resorption of Litter	0	0	1	0
	Mean No. Corpora Lutea	16.4	16.6	15.4	14.1**
	Mean No. Implantations	15.1	15.5	12.1	13.0*
	Mean % Preimplantation Loss	7.6	5.8	22.0	7.6
	Mean No. Live Conceptuses	14.7	14.8	11.2*	12.4*
	Mean No. Resorptions	0.0	0.0	0.0	0.0

APPENDIX 4 (cont.)

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2.6.7.12A Reproductive and Developmental Toxicity: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats

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2.6.7.12A Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation Report Title: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats (continued)

Daily Dose (mg/kg)		0 (Control)	6	60	600
Treated Females/Treated Males	Mean No. Placental remnants	0.4	0.7	0.8	0.5
	Mean No. Dead Embryos	0.0	0.1	0.1	0.1
	Mean % Postimplantation Loss	2.8	5.0	12.7**	4.8
Untreated Females/Treated Males	No. Evaluated	20	NE	20	20
	Mean No. Days Prior to Mating	2.0	NE	2.7	2.1
	No. of Females Sperm Positive	20	NE	19	19
	No. of Pregnant Females	18	NE	18	18
	No. Aborted or with Total Litter Resorption	0	NE	0	0
	Mean No. Corpora Lutea	16.7	NE	16.3	15.8
	Mean No. Implantations	15.8	NE	14.1	14.1
	Mean % Preimplantation Loss	5.3	NE	13.7	10.9
	Mean No. Live Conceptuses	14.6	NE	12.8	13.1
	Mean No. Resorptions	0.0	NE	0.0	0.1
	Mean No. Placental Remnants	1.1	NE	1.2	0.8
	Mean No. Dead Embryos	0.1	NE	0.1	0.2
Mean % Postimplantation Loss	7.3	NE	10.7	7.9	

- indicates No noteworthy findings; Dunnett's Test: *, $p \leq 0.05$; **, $p \leq 0.01$; NA, not applicable; NE, not examined.

(a) From Study/Report No: 2742/FE/M-11-38.

(b) After 9 weeks of dosing. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(c) After first dose only. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(d) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(e) Twenty males were alive; however, 9 females died prior to initiation of the mating period.

(f) Dead animals.

(h) At the end of the pre-mating or gestation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(i) Gestation Day 3, only. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

2.6.7.12B Reproductive and Developmental Toxicity Study: Effects of TAK-375 on Fertility
and Early Embryonic Development to Implantation in Rats – Supplemental Study I (Plasma Concentration)

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2.6.7.12B Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation Report
Title: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats–Supplemental
Study I (Plasma Concentration)

Design similar to ICH 4.1.1?:	No	Duration of Dosing:	Single dose	Test Article	TAK-375
Species/Strain:	CD(SD)IGS Rats	Day of Mating:	-	Study/Report No:	2742/FE/M-11-38
Initial Age:	M: 6 and 11 weeks; F: 6 and 12 weeks	Day of C-Section:	-	GLP Compliance:	Yes
Date of First Dose:	10 Jun 98	Method of Administration:	Gavage		
		Vehicle/Formulation:	0.5 w/v% methylcellulose solution		

Special Features: Study conducted to determine plasma concentrations in 6 and 11-week old rats (a)

Daily Dose (mg/kg)	6	60	600	60	600
Males					
Age (weeks)	11	11	11	6	6
Toxicokinetics					
C _{max} (µg/mL)	0.40	8.74	28.66	8.38	27.23
AUC (µg·hr/mL)	0.6	26.3	230.5	15.3	178.2
No. Evaluated	4	4	4	4	4
No. Died or Sacrificed Moribund	0	0	0	0	0
Clinical Observations					
Crouching	-	-	2	-	2
Decrease in spontaneous activity	-	-	3	-	4
Ataxic gait	-	-	-	-	1
Necropsy Observations	-	-	-	-	-

APPENDIX 4 (cont.)

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2.6.7.12B Reproductive and Developmental Toxicity Study: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats – Supplemental Study I (Plasma Concentration)

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**2.6.7.12B Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation Report
Title: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats–Supplemental Study I (Plasma Concentration) (continued)**

Daily Dose (mg/kg)		6	60	600	60	600
Females	Age (weeks)	12	12	12	6	6
	Toxicokinetics					
	Cmax (µg/mL)	1.24	15.13	78.50	11.30	51.53
	AUC (µg·hr/mL)	1.3	67.8	1253.1	38.3	601.9
	No. Evaluated	4	4	6	4	4
	No. Died or Sacrificed Moribund	0	0	2	0	0
	Clinical Observations					
	Creeping	-	-	2	-	1
	Crouching	-	-	2	-	-
	Decreased spontaneous activity	-	-	1	-	2
	Ataxic gait	-	-	3	-	2
	Lying on side	-	-	1	-	-
	Necropsy Observations					
	Discolored liver	0	0	1 (b)	0	1
	Discolored kidney	0	0	0	0	1
	Lung congestion	0	0	1 (c)	0	0
	Enlarged adrenal glands	0	0	1 (c)	0	0
	Discolored spleen	0	0	1 (b)	0	0
	Petechia in Stomach	0	0	1 (b)	0	0

- indicates No noteworthy findings.

(a) This study was conducted to evaluate and compare plasma levels obtained for 6-week and 11- or 12-week old animals. Reproductive parameters were not assessed in this study.

(b) Noted for animal that was sacrificed moribund.

(c) Noted for animal found dead.

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2.6.7.12C Reproductive and Developmental Toxicity: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats – Supplemental Study II

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2.6.7.12C Reproductive and Developmental Toxicity, Fertility and Early Embryonic Development to Implantation Report
Title: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats–Supplemental Study II

Design similar to ICH 4.1.1?	Yes	Duration of Dosing:	M: 7 weeks (4 weeks pre-mating through day before necropsy) F: 2 weeks pre-mating through GD 6	Test Article:	TAK-375
Species/Strain:	CD(SD)IGS Rat	Day of Mating:	Day 0	Study/Report No:	2782/FE/M-11-74
Initial Age:	M: 11 weeks; F: 10 weeks	Day of C-Section:	Gestation Day 13	GLP Compliance:	Yes
Date of First	28 Jul 98	Method of Administration:	Gavage		
Special Features:	None	Vehicle/Formulation:	0.5 w/v% methylcellulose solution		
No Observed Adverse Effect Level					
F0 Males:	60 mg/kg/day				
F0 Females:	20 mg/kg/day				
F1 Litters:	200 mg/kg/day				

Daily Dose (mg/kg)		0 (Control)	20	60	200
Males	No. Evaluated	20	20	20	20
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations				
	Creeping	-	-	-	2
	Crouching	-	-	-	3
	Decrease in spontaneous activity	-	-	-	12
	Ataxic gait	-	-	-	3
	Salivation	-	-	1	17
	Necropsy Observations				
	Body Weight (%) (a)	547 g	0	-1.1	-1.1
	Food Consumption (%) (b)	28 g	+3.6	0	0
	Organ Weight (%) (c)	-	-	-	-
	Mean No. Days Prior to Mating	2.9	4.1	2.8	2.2
	No. of Males That Mated	20	20	20	20
No. of Fertile Males	20	19	19	17	

APPENDIX 4 (cont.)

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 2.6.7.12C Reproductive and Developmental Toxicity: Effects of TAK-375 on Fertility
 and Early Embryonic Development to Implantation in Rats – Supplemental Study II

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2.6.7.12C Reproductive and Developmental Toxicity, Fertility and Early Embryonic Development to Implantation Report
 Title: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats–Supplemental
 Study II (continued)

Daily Dose (mg/kg)		0 (Control)	20	60	200	
Females	No. Evaluated	20	20	20	20	
	No. Died or Sacrificed Moribund	0	0	0	0	
	Clinical Observations					
	Creeping	-	-	-	4	
	Crouching	-	-	-	2	
	Decreased spontaneous activity	-	-	-	12	
	Ataxic gait	-	-	-	9	
	Decrease in fecal pellets	-	-	-	4	
	Salivation	-	-	-	18	
	Necropsy Observations					
	Premating Body Weight (%) (d)	267 g	-1.9	-1.1	-3.7	
	Gestation Body Weight (%) (d)	337 g	-1.8	-0.3	-4.5*	
	No. Evaluated	20	20	20	20	
	Premating Food Consumption (%) (d)	18 g	+11.1	0	0	
	Gestation Food Consumption (%) (d)	27 g	0	+7.4	0	
	No. Irregular Estrous Cycles (%)	3 (15.0)	1 (5.0)	6 (30.0)	6 (30.0)	
	Mean No. Days Prior to Mating	2.9	4.1	2.8	2.2	
	No. of Females Sperm Positive	20	20	20	20	
	No. of Pregnant Females	20	19	19	17	
	No. Aborted or with Total Resorption of Litter	0	0	0	0	
	Mean No. Corpora Lutea	16.2	16.3	16.9	15.8	
	Mean No. Implantations	14.7	15.7	16.2*	15.1	

2.6.7.12C Reproductive and Developmental Toxicity, Fertility and Early Embryonic Development to Implantation Report
Title: Effects of TAK-375 on Fertility and Early Embryonic Development to Implantation in Rats–Supplemental
Study II (continued)

Daily Dose (mg/kg)		0 (Control)	20	60	200
Females	No. Evaluated	20	20	20	20
	Mean % Preimplantation Loss	9.3	3.7	4.4	4.3
	Mean No. Live Conceptuses	14.1	14.8	14.9	14.1
	Mean No. Resorptions	0.0	0.0	0.0	0.0
	Mean No. Placental Remnants	0.5	0.8	1.3*	1.1
	Mean No. Dead Embryos	0.1	0.1	0.0	0.0
	Mean % Postimplantation Loss	4.1	5.2	7.7	7.1

- indicates No noteworthy findings; Dunnett's Test: *, $p \leq 0.05$.

(a) At the end of the treatment period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(b) After the first dose. For controls, group means are shown. For treated groups, percent differences from the controls are shown. Statistical significance is based on the actual data (not the percent differences).

(c) For controls, group means are shown. For treated groups, percent differences from the controls are shown. Statistical significance is based on the actual data (not the percent differences).

(d) At the end of the premating or gestation period. For controls, group means are shown. For treated groups, percent differences from the controls are shown. Statistical significance is based on the actual data (not the percent differences).

APPENDIX 4 (cont.)

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2.6.7.13A Reproductive and Developmental Toxicity Study: Effects of TAK-375 on Embryo-Fetal Development in Rats

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2.6.7.13A Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development Report Title:
Effects of TAK-375 on Embryo-Fetal Development in Rats

Design similar to ICH 4.1.3.?:	Yes	Duration of Dosing:	G6-G17	Test Article:	TAK-375	
Species/Strain:	\ CD(SD)IGS Rats	Day of Mating:	Day 0	Study/Report No:	2701/TE//M-11-36	
Initial Age:	12 Weeks	Day of C-Section:	G20	GLP Compliance:	Yes	
Date of First Dose:	6 Apr 98	Method of Administration:	Gavage			
Special Features:	None	Vehicle/Formulation:	0.5 w/v% methylcellulose solution			
No Observed Adverse Effect Level						
F0 Females: 40 mg/kg						
F1 Litters: 40 mg/kg						
Daily Dose (mg/kg)		0 (Control)	10	40	150	600
Dams	Toxicokinetics: (a)					
	TAK-375 Cmax (µg/mL)	NA	NE	3.305	8.011	NE
	M-II (2S, 8S) Cmax (µg/mL)	NA	NE	1.138	1.766	NE
	M-II (2R, 8S) Cmax (µg/mL)	NA	NE	0.428	0.715	NE
	TAK-375; AUC (µg·h/mL)	NA	NE	17.790	81.595	NE
	M-II (2S, 8S); AUC (µg·h/mL)	NA	NE	10.772	17.373	NE
	M-II (2R, 8S); AUC (µg·h/mL)	NA	NE	3.719	7.489	NE
	No. Pregnant	20	20	20	20	16
	No. of Deaths	0	0	0	0	1
	No. Aborted or with Total Resorption of Litter	0	0	0	0	1
	Clinical Observations					
	Salivation	-	-	-	10	14
	Lying on side	-	-	-	-	2
	Decrease in spontaneous activity	-	-	-	-	11
	Ataxic gait	-	-	-	-	8
	Decrease in fecal pellets	-	-	-	-	9
	Necropsy Observations					
	Enlarged adrenal glands	0	0	0	0	2

**2.6.7.13A Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development Report Title:
Effects of TAK-375 on Embryo-Fetal Development in Rats (continued)**

Daily Dose (mg/kg)		0 (Control)	10	40	150	600
Dams	Organ weight (%) (b)					
	Liver (g)	14.76	+3.0	+3.7	+9.4*	+23.3**
	Adrenal glands (mg)	69.2	+1.2	-4.3	+6.9	+30.8**
	Carcass (g)	225	+1.8	+1.3	+1.8	-8.9**
	Body Weight (%) (b)					
	Day 18	356 g	+2.0	+1.1	+0.8 (c)	-11.0**
	Day 20	385 g	+2.1	+1.0	+1.3	-9.9**
	Food Consumption (%) (b)					
	Day 16	28 g	+3.6	+7.1	+7.1	-25.0**
	Mean No. of Corpora Lutea	15.9	16.2	16.6	15.7	15.9
	Mean No. of Implantations	14.6	15.4	14.9	14.9	14.7
	Mean (%) Preimplantation Loss	7.7	4.3	8.5	4.6	6.0
Litters	No. Litters Evaluated	20	20	20	20	14
	No. Live Fetuses	284	298	287	286	195
	Mean No of Live Fetuses	14.2	14.9	14.4	14.3	13.0
	Mean No. of Resorptions	0.0	0.0	0.0	0.0	0.9
	Mean No. of Placental Remnants	0.4	0.5	0.5	0.6	0.8
	Mean No. of Dead Fetuses	0.0	0.0	0.0	0.0	0.1
	Mean (%) Postimplantation Loss	2.4	3.1	3.5	4.2	12.2
	Sex Ratios (% males)	49.6	45.8	48.2	45.3	55.7
	Mean Fetal Body Weight (g)					
	Male	3.50	3.45	3.46	3.38	2.87**
	Female	3.33	3.27	3.23	3.22	2.71**
	Placental Abnormalities (%)	0	0.3	0	0	0
External Abnormalities (%)	0.4	0.6	0	0	2.1	

APPENDIX 4 (cont.)

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2.6.7.13A Reproductive and Developmental Toxicity Study: Effects of TAK-375 on Embryo-Fetal Development in Rats

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2.6.7.13A Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development Report Title:
Effects of TAK-375 on Embryo-Fetal Development in Rats (continued)

Daily Dose (mg/kg)		0 (Control)	10	40	150	600
Litters	Cyst (External genitalia)					
	No. of Fetuses (%)	0	0	0	0	3 (1.5*)
	No. of Litters (%)	0	0	0	0	2 (14.3)
	Visceral Abnormalities (%)	0	0	0.7	1.8	12.8**
	Diaphragmatic hernia (%)					
	No. of Fetuses (%)	0	0	0	2 (1.2)	12 (11.9**)
	No. of Litters (%)	0	0	0	2 (10)	6 (42.9)
	Visceral Variations (%)	0	1.7	0.6	3.2	2.0
	Skeletal Abnormalities (%)	1.0	0	0	0.7	2.3
	Skeletal Variations (%)	12.1	12.8	19.5	23.3	51.8**
	Irregularly shaped scapula					
	No. of Fetuses (%)	0	1(0.6)	1(0.8)	8(6.1)	27 (29.8**)
	No. of Litters (%)	0	1(5)	1(5)	5(25)	9 (64.3)
	Lumbar rib					
	No. of Fetuses (%)	10(7.7)	13(9.3)	21(15.6)	20(14.8)	22 (24.9)
	No. of Litters (%)	7(35)	7(35)	13(65)	12(60)	10 (71.4)
	Mean No. of Sacro-Caudal Vertebrae Ossified	7.8	7.7	7.8	7.9	7.2

- indicates No noteworthy findings, G, Gestation Day; NA, not applicable; NE, not examined; Dunnett's Test *, p≤0.05, **, p≤0.01.

(a) Gestation Day 17; from Report No. TAK-375/M-11-173.

(b) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(c) A statistically significant decrease in body weight gain was noted for Gestation Days 6 to 8; however, there were no statistically significant differences in mean body weight data.

2.6.7.13B Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development Report Title: Effects of
Intravenously Injected TAK-375 M-II (2S,8S) on Embryo-Fetal Development in Rats

Design similar to ICH 4.1.3.7:	Yes	Duration of Dosing:	G6-G17	Test Article	TAK-375
Species/Strain:	\ CD(SD)IGS Rats	Day of Mating:	Day 0	Study/Report No:	3117/TE/M-11-187
Initial Age:	12 Weeks	Day of C-Section:	G20	GLP Compliance:	Yes
Date of First Dose:	30 Aug 99	Method of Admin.:	Intravenous		
Special Features:	None	Vehicle/Formulation:	30 vol% PEG400		
No Observed Adverse Effect Level:					
F0 Females:	30 mg/kg				
F1 Litters:	30 mg/kg				

Daily Dose (mg/kg)		0 (Control)	10	30
Dams	Plasma concentration ($\mu\text{g/mL}$) (a)			
	M-II (2S,8S) C(5 min)	NE	13.633	19.575
	M-II (2R,8S) C(5 min)	NE	0.013	0.057
	M-II (2S,8S) C(15 min)	NE	7.173	22.806
	M-II (2R,8S) C(15 min)	NE	0.033	0.103
	M-II (2S,8S) C(4 hr)	NE	0.055	0.182
	M-II (2R,8S) C(4 hr)	NE	0.00	0.002
	No. Pregnant	16	17	17
	No. Died or Sacrificed Moribund	0	0	0
	No. Aborted or with Total Resorption of Litter	0	0	0
	Clinical Observations	-	-	-
	Necropsy Observations	-	-	-
	Body Weight (%)	-	-	-
	Food Consumption (%)	-	-	-
	Mean No. of Corpora Lutea	16.5	16.2	15.8
	Mean No. of Implantations	15.5	15.4	15.1
	Mean Preimplantation Loss (%)	5.1	4.8	4.6

APPENDIX 4 (cont.)

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 2.6.7.13B Reproductive and Developmental Toxicity: Effects of Intravenously Injected TAK-375 M-II (2S,8S)
 on Embryo-Fetal Development in Rats

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2.6.7.13B Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development Report Title: Effects of
 Intravenously Injected TAK-375 M-II (2S,8S) on Embryo-Fetal Development in Rats (continued)

Daily Dose (mg/kg)		0 (Control)	10	30
Litters	No. Litters Evaluated	16	17	17
	No. Live Fetuses	233	245	228
	Mean No. of Live Fetuses	14.6	14.4	13.4
	Mean No. of Resorptions	0.0	0.0	0.0
	Mean No. of Placental Remnants	0.9	0.9	1.6
	Mean No. of Dead Fetuses	0.0	0.0	0.1
	Mean Postimplantation Loss (%)	5.8	6.2	11.0
	Sex Ratios (% males)	50.4	48.5	44.6
	Mean Placental Weight (mg)			
	Male	434	437	479
	Female	433	418	451
	Mean Fetal Body Weight (g)			
	Male	3.44	3.43	3.52
	Female	3.23	3.28	3.30
	Placental Abnormalities (%)	0 (0.0)	0 (0.0)	1 (0.4)
	External Abnormalities (%)	0 (0.0)	0 (0.0)	0 (0.0)
	Visceral Abnormalities (%)	0 (0.0)	NE	1 (1.0)
	Visceral Variations (%)	0 (0.0)	NE	3 (2.5)
	Skeletal Abnormalities (%)	0 (0.0)	NE	0 (0.0)
	Skeletal Variations (%)	20 (18.9)	NE	18 (18.1)
	Mean No. of Sacro-Caudal Vertebrae Ossified	8.0	NE	7.9

- indicates No noteworthy findings.; G, Gestation Day; NE, not examined.
 M-II (2S,8S) is a metabolite of TAK-375.
 (a) Gestation Day 17.

2.6.7.13C Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development Report Title: Effects of TAK-375 on Embryo-Fetal Development in Rabbits

Design similar to ICH 4.1.3.2: Yes	Duration of Dosing: G6-G18	Test Article: TAK-375		
Species/Strain: \ JW Rabbit	Day of Mating: Day 0	Study/Report No: 2756/TE/M-11-103-1		
Initial Age: 16 Weeks	Day of C-Section: G28	GLP Compliance: Yes		
Date of First Dose: 22 June 98	Method of Administration: Gavage			
Special Features:	Vehicle/Formulation: 0.5 w/v% methylcellulose solution			
No Observed Adverse Effect Level:				
F0 Females: 60 mg/kg/day				
F1 Litters: 300 mg/kg/day				
Daily Dose (mg/kg)	0 (Control)	12	60	300
Does				
Toxicokinetics (a)				
TAK-375 C _{max} (µg/mL)	ND	1.27	23.96	58.01 (b)
	ND	ND	ND	35.514 (c)
M-I C _{max} (µg/mL)	ND	ND	ND	49.197 (c)
M-II C _{max} (µg/mL)	ND	ND	ND	3.368 (c)
M-III C _{max} (µg/mL)	ND	ND	ND	5.233 (c)
M-IV C _{max} (µg/mL)	ND	ND	ND	2.668 (c)
TAK-375: AUC (µg·h/mL)	ND	4.7	22.0	111.5 (b)
	ND	ND	ND	71.463 (c)
M-I AUC (µg·h/mL)	ND	ND	ND	139.290 (c)
M-II AUC (µg·h/mL)	ND	ND	ND	23.847 (c)
M-III AUC (µg·h/mL)	ND	ND	ND	21.203 (c)
M-IV AUC (µg·h/mL)	ND	ND	ND	16.873 (c)
No. Pregnant	19	19	20	18
No. Died or Sacrificed Moribund	0	0	0	0
No. Aborted or with Total Resorption of Litter	0	0	1	3

APPENDIX 4 (cont.)

2.6.7.13C Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development Report Title: Effects of TAK-375 on Embryo-Fetal Development in Rabbits (continued)

Daily Dose (mg/kg)		0 (Control)	12	60	300
Does	Clinical Observations				
	Decrease in spontaneous activity	-	-	-	18
	Creeping	-	-	-	18
	Rigidity	-	-	-	10
	Lying on one side	-	-	-	11
	Myosis	-	-	-	15
	Convulsions	-	-	-	2
	Decrease in feces	2	1	4	16
	Necropsy Observations	-	-	-	-
	Organ weight (%) (d)	-	-	-	-
	Body Weight (%) (d)				
	Day 18	3.56 kg	+1.1	+0.3	-6.7*
	Day 28	3.73 kg	+1.1	+1.1	-1.6
	Food Consumption (%) (d)				
	Day 18	151 g	+2.6	-11.3	-37.7**
	Mean No. of Corpora Lutea	10.8	10.8	10.9	10.1
	Mean No. of Implantations	8.0	8.7	9.2	8.4
Mean Preimplantation Loss (%)	26.3	19.0	15.9	16.6	
Litters	No. Litters Evaluated	19	19	19	15
	No. Live Fetuses	141	146	163	117
	Mean No. of Live Fetuses	7.4	7.7	8.6	7.3
	Mean No. Resorptions	0.0	0.0	0.0	0.0
	Mean No. Placental Remnants	0.3	0.6	0.3	0.6
	Mean No. Dead Fetus	0.3	0.4	0.3	0.5
	Mean Postimplantation Loss (%)	6.4	11.2	8.3	17.1
	Sex Ratios (% males)	48.0	50.6	49.1	43.6

2.6.7.13C Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development Report Title: Effects of TAK-375 on Embryo-Fetal Development in Rabbits (continued)

Daily Dose (mg/kg)	0 (Control)	12	60	300
Litters				
Mean Fetal Body Weight (g)				
Male	39.54	38.92	38.22	37.60
Female	37.42	37.60	36.35	36.47
Placental Abnormalities (%)	5.1	1.3	3.5	1.9
External Abnormalities (%)	0.4	0.0	1.5	0.0
Visceral Abnormalities (%)	0.4	0.0	0.0	0.8
Visceral Variations (%)	0.0	1.1	0.0	0.0
Skeletal Abnormalities (%)	1.8	NE	NE	0.0
Skeletal Variations (%)	25.9	NE	NE	40.7
Mean. No. of Sacro-Caudal Vertebrae ossified	19.1	NE	NE	19.0

- indicates No noteworthy findings; G, Gestation Day; Dunnett's Test: *, $p \leq 0.05$; **, $p \leq 0.01$; NE, not examined; ND, not determined.

(a) Gestation Day 18.

(b) Only plasma levels of TAK-375 were measured concurrent with the embryo-fetal development study.

(c) Results from Report No. TAK-375/M-11-188.

(d) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not the percent differences).

APPENDIX 4 (cont.)

Ramelteon

2.6.7.14 Reproductive and Developmental Toxicity: Effects of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function in Rats

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2.6.7.14 Reproductive and Developmental Toxicity

Test Article: TAK-375

Effects on Pre- and Postnatal Development Including Maternal Function

Report Title: Effects of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function in Rats

Design similar to ICH 4.1.2?:	Yes	Duration of Dosing:	G6 to L21	Test Article:	TAK-375
Species/Strain:	\ CD(SD)IGS Rats	Day of Mating:	Day 0	Study/Report No.:	300319/M-11-00271.001
Initial Age:	13 Weeks	Method of Administration:	Gavage	GLP Compliance:	Yes
Date of First Dose:	6 Mar 00	Vehicle/Formulation:	0.5 w/v% methylcellulose solution		
		Litters Culled/Not Culled:	Culled to 4/sex/litter		
Special Features:	None				
No Observed Adverse Effect Level:	F0 Females and F1 Animals: 30 mg/kg/day; F0 maternal reproductive function: 100 mg/kg/day				
Daily Dose (mg/kg)		0 (Control)	30	100	300
F0 Females	No. Pregnant	20	19	20	20
	No. Died or Sacrificed Moribund	0	0	0	0
	No. Aborted or with Total Resorption of Litter	0	0	0	0
	Clinical Observations				
	Decrease in locomotor activity	-	-	-	18
	Salivation	-	-	19	20
	Necropsy Observations	-	-	-	-
	Organ Weight (%) (a)				
	Adrenal glands (mg)	65.8	+5.6	+9.4	+28.1**
	Gestation Body Weight (%) (b)	416 g	-0.5	-1.9	-6.7**
	Lactation Body Weight (%) (b)	328 g	-1.5	-3.4	-5.2**
	Gestation Food Consumption (%)	-	-	-	↓ (c)
	Lactation Food Consumption (%) (d)	64 g	-1.6	-6.3*	-12.5**
	Mean Duration of Gestation (days)	22.0	22.3*	22.2	22.2
	Abnormal Parturition	-	-	-	-

2.6.7.14 Reproductive and Developmental Toxicity

Test Article: TAK-375

Effects on Pre- and Postnatal Development Including Maternal Function

Report Title: Effects of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function in Rats (continued)

Daily Dose (mg/kg)		0 (Control)	30	100	300
F1 Litters (Prewearing)	No. Litters Evaluated	20	19	20	20
	Mean No. of Implantations	15.6	15.2	15.6	15.1
	Mean No. Live offsprings/Litter	14.1	13.8	14.0	13.5
	Mean No. of Stillbirths	0.6	0.3	0.5	0.4
	Birth Index (%)	90.1	90.9	90.3	90.0
	Viability Index (Day 4) (%)	98.4	91.7	97.6	83.1
	Weaning Index (%)	100.0	100.0	100.0	100.0
	No. of Total Litter Losses	0	1	0	3
	Body Weight (%) (e)				
	Male	53.7 g	-0.4	-6.5*	-13.2**
	Female	52.0 g	+1.0	-6.0*	-12.9**
	Sex Ratios [Male/(Male+Female)]	0.54	0.50	0.53	0.50
	Pup Clinical Signs	-	-	-	-
	Morphological Differentiations				
	Eruption of lower incisors (%)				
	Day 10	32	27	20	15*
	Day 11	73	71	67	51*
	Reflex Functions				
	Righting Reflex (%)				
	Day 6	75	78	68	41**
Day 7	98	94	91	83*	
Negative Geotaxis (%)					
Day 11	64	92**(a)	79	81	
Day 12	88	99**(a)	96 *(a)	94	

APPENDIX 4 (cont.)

Ramelteon

2.6.7.14 Reproductive and Developmental Toxicity: Effects of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function in Rats

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2.6.7.14 Reproductive and Developmental Toxicity
Effects on Pre- and Postnatal Development Including Maternal Function
Report Title: Effects of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function in Rats (continued)

Test Article: TAK-375

Daily Dose (mg/kg)		0 (Control)	30	100	300
F1 Litters (Prewearing)	Pup Necropsy Observations				
	Diaphragmatic hernia				
	No. of Males (%)	0	0	0	2 (6.3)
	No. of Females (%)	0	0	0	1 (3.1)
	Skeletal Abnormalities in Weanlings (%)	1.3	1.4	1.3	1.6
	Skeletal Variations in Weanlings (%)	15.0	11.1	16.3	25.0
F1 Males (Postweaning)	No. Evaluated Postweaning Per Litter	20	18	20	17
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	-	-	-	-
	Necropsy Observations	-	-	-	-
	Body Weight (%) (f)	462 g	-1.9	-5.6*	-10.0**
	Cleavage of the Balanopreputial Gland	-	-	-	-
	Emotional examination				
	Latency (sec.)	14.2	14.5	19.0	30.1
	Learning ability	-	-	-	-
	Mean No. Days Prior to Mating	2.8	4.1	3.5	2.4
	No. of Males that Mated	20	18	20	17
	No. of Fertile Males	19	17	20	17
	Copulation index (%)	100.0	100.0	100.0	100.0
	Fertility index (%)	95.0	94.4	100.0	100.0

2.6.7.14 Reproductive and Developmental Toxicity

Test Article: TAK-375

Effects on Pre- and Postnatal Development Including Maternal Function

Report Title: Effects of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function in Rats (continued)

Daily Dose (mg/kg)		0 (Control)	30	100	300
F1 Females (Postweaning)	No. Evaluated Postweaning	20	18	20	17
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	-	-	-	-
	Necropsy Observations	-	-	-	-
	Premating Body Weight (%) (f)	270 g	+0.7	-0.4	-1.1
	Gestation Body Weight (%) (h)	429 g	+1.4	+0.7	+1.9
	Lactation Body Weight (%) (h)	332 g	+2.1	+0.9	+3.6
	Vaginal opening	-	-	-	-
	Emotional examination				
	Latency (sec.)	9.7	9.2	18.9	32.9*
	Learning ability	-	-	-	-
	Number of Estrous Cases before Mating (14 days)	3.5	3.4	3.5	3.2
	Mean No. Days Prior to Mating	2.8	4.1	3.5	2.4
	No. of Females that Mated	20	18	20	17
	No. of Pregnant females	19	17	20	17
	Copulation index (%)	100.0	100.0	100.0	100.0
	Fertility index (%)	95.0	94.4	100.0	100.0
	Mean No. Implantations	12.4	13.5	14.1	13.4
	Mean Duration of Gestation (Days)	22.2	22.1	22.4	22.3
	Abnormal Parturition	-	-	-	-

APPENDIX 4 (cont.)

Ramelteon

2.6.7.14 Reproductive and Developmental Toxicity: Effects of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function in Rats

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2.6.7.14 Reproductive and Developmental Toxicity
Effects on Pre- and Postnatal Development Including Maternal Function
Report Title: Effects of Orally Administered TAK-375 on Pre- and Postnatal Development, Including Maternal Function in Rats (continued)

Test Article: TAK-375

Daily Dose (mg/kg)		0 (Control)	30	100	300
F2 Litters	Mean No. Live offsprings/Litter	11.5	12.1	12.8	11.9
	Mean No. of Stillbirths	0.2	0.5	0.2	0.6
	Birth Index (%)	91.2	89.5	90.8	89.0
	Viability index (Day 4) (%)	93.2	99.0	98.6	91.1
	Weaning index (%)	100.0	100.0	96.3	95.3
	Body Weights				
	Male	-	-	-	-
	Female	-	-	-	-
	Sex Ratios [Male/(Male+Female)]	0.59	0.54	0.51	0.53
	Clinical Observations	-	-	-	-
	External Anomalies (%)	0.0	0.0	0.0	0.0
	Necropsy Findings	-	-	-	-

- indicates No noteworthy findings; Dunnett's Test *, $p \leq 0.05$; **, $p \leq 0.01$; ↓, decrease; G, Gestation Day; L, Lactation Day.

(a) For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(b) At the end of the gestation or lactation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(c) Food consumption significantly decreased (14.8% to 28.6% of control) on Gestation Days 6 to 14.

(d) At Day 14 of the lactation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(e) At weaning (Day 21). For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(f) At Week 11. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

(h) At the end of the gestation or lactation period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on the actual data (not the percent differences).

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this page is the manifestation of the electronic signature.**

/s/

Adam Wasserman
6/22/05 05:08:54 PM
PHARMACOLOGIST

R. Daniel Mellon
6/22/05 05:24:57 PM
PHARMACOLOGIST
I concur with Dr. Wasserman's recommendations.

MEMORANDUM
DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
CONTROLLED SUBSTANCE STAFF

Date: June 7, 2005

To: Bob Rappaport, M.D., Director
Division of Anesthetic, Critical Care and Addiction Drug Products
(HFD-170)

Through: Deborah B. Leiderman, M.D., Director
Michael Klein, Ph.D., Team Leader
Controlled Substance Staff (HFD-009)

From: Katherine Bonson, Ph.D., Pharmacologist
Controlled Substance Staff (HFD-009)

Subject: Consult on abuse potential for NDA review
Ramelteon []
NDA 21-782
Treatment of [] insomnia
Sponsor: Takeda Pharmaceuticals, Lincolnshire, IL

Background:

The Division of Anesthetic, Critical Care and Addiction Drug Products (HFD-170) consulted CSS regarding the abuse potential of ramelteon [], previously known as TAK-375. Ramelteon is a highly selective melatonin-1 (ML-1) receptor agonist that is being proposed for use in the treatment of chronic and transient insomnia. Ramelteon has been tested in 42 clinical trials at doses ranging from 0.3 to 160 mg. The recommended therapeutic dose will be 8 mg, taken orally before bedtime.

Some medications approved for the treatment of sleep disorders are controlled substances under the Controlled Substances Act (CSA). The Sponsor proposes that ramelteon not be controlled under the CSA, citing the results from non-clinical studies, clinical trials and human abuse potential studies as support for their position that ramelteon lacks abuse potential and should be approved for marketing as a non-scheduled drug.

Conclusions and Recommendations:

Based upon review of data provided by the Sponsor, CSS concludes that ramelteon does not have abuse liability similar to that of other scheduled drugs indicated for the treatment of sleep disorders.

The most salient finding for this conclusion is that ramelteon did not produce rewarding properties in a human laboratory abuse liability study, as evaluated by a battery of subjective measures, at doses that are 2, 10 and 20 times higher than the recommended therapeutic dose. These data demonstrated that the abuse potential of ramelteon is similar to that of placebo.

Ramelteon did not produce any signals from animal behavioral studies indicating that the drug produces rewarding effects. Monkeys do not self-administer ramelteon and it does not induce a conditioned place preference in rats. There is no generalization between the Schedule IV benzodiazepine, midazolam, and ramelteon. Ramelteon did not affect rotorod performance, an indicator of disruption of motor function, nor did it potentiate the ability of the Schedule IV benzodiazepine, diazepam, to interfere with rotorod performance.

Discontinuation of ramelteon in animals or in humans after chronic administration did not produce withdrawal signs. Ramelteon does not appear to produce physical dependence.

Finally, there is little evidence that ramelteon may be used to incapacitate an unwitting individual for the purpose of committing malicious crimes (such as sexual assault) against that individual. Although ramelteon is freely soluble in ethanol and produces initial sedative effects within 1.5 hr after oral administration in clinical patients with insomnia, there is no evidence that ramelteon produces disruptive behavioral effects that would render an individual defenseless or liable to physical assault. Ramelteon does produce an additive effect to the sedation produced by ethanol. However, human studies with ramelteon at doses up to 20 times those recommended for therapeutic use did not induce changes in cognition or motor performance, nor was there any evidence of amnesia or memory impairment.

I. Summary of Data Related to Abuse Potential from Human Studies

A. Human Laboratory Abuse Potential Study

Ramelteon did not produce rewarding properties in a human laboratory abuse liability study, conducted in polydrug abusers with at least one experience with a sedative-hypnotic drug in the past year. At doses of 16, 80 or 160 mg (p.o.) (representing 2, 10 and 20 times the recommended therapeutic dose), ramelteon did not produce statistically significant increases in subjective responses to "good drug effect", "drug liking", "drug strength", "street value" and "willing to pay". In contrast, the Schedule IV

benzodiazepine, triazolam, produced statistically significant increases in each of these subjective measures. Neither ramelteon or triazolam produced adverse events indicative of euphoria. These data suggest that the abuse potential of ramelteon is similar to that of placebo.

B. Adverse Events in Clinical Efficacy Studies Related to Abuse Liability

The most common adverse event reported by patients in clinical trials with ramelteon was somnolence (33% at 16 mg dose, twice the recommended therapeutic dose). Headache was reported by 64% of those patients exposed to 64 mg of ramelteon (8 times the recommended therapeutic dose). No euphoria was reported by patients, even at doses of ramelteon up to 128 mg (16 times the recommended therapeutic dose). There have been no reports of overdose, nor any reports of safety issues at doses up to 160 mg (20 times the recommended therapeutic dose).

C. Physical Dependence Evaluated in Clinical Efficacy Studies

Ramelteon did not produce rebound insomnia following drug discontinuation in three clinical efficacy studies in adults, one of which evaluated elderly patients. Additionally, ramelteon did not produce any other signs of a withdrawal syndrome, as evaluated by the Benzodiazepine Withdrawal Syndrome Questionnaire.

II. Summary of Data Related to Abuse Potential from Preclinical Studies

A. Receptor Binding

Ramelteon is a highly selective melatonin-1 (ML-1) receptor agonist. It does not have a receptor binding profile that is similar to any known drugs of abuse, nor does it bind significantly to any major or minor neurotransmitter system in the brain with the exception of the melatonin receptor. The mechanism of action of ramelteon is not well understood.

B. Metabolites

Four metabolites of ramelteon were identified in rats and monkeys (M-I, M-II, M-III and M-IV), all of which are present in humans. The major metabolite is M-I and the secondary metabolite is M-III, with plasma levels that are between 1/2 to 1/3 those of M-I. M-II does not bind to any receptors associated with known drugs of abuse.

C. Behavioral Studies

The preclinical behavioral studies with ramelteon include self-administration, conditioned place preference and drug discrimination.

Self-Administration

Two self-administration studies were conducted.

In the first study, ramelteon did not induce self-administration (< 10 injections/2-hour session) at a range of doses (0.01 - 0.3 mg/kg/injection, i.v.) in 3 of 4 monkeys trained to self-administer the sedative methohexital (0.1 mg/kg/injection, i.v.; mean of 100 injections/2-hour session). This rate of ramelteon injection was similar to that of saline and suggests that ramelteon does not have significant rewarding effects. However, 1 of the 4 monkeys did self-administer ramelteon at the three lower doses tested (mean of 90-100 injections/session), but at much lower rate when the highest dose was administered (mean of ~50 injections/ session). The lack of a dose-response effect in this single monkey suggests that the results may be anomalous.

Given the difficulty in interpreting the first study, a second study was conducted. Ramelteon was not self-administered at a rate that differed from that for vehicle when tested at 0.025 - 0.4 mg/kg/infusion (i.v.) when given access to the drug in a 2-hour session. A similar lack of self-administration was seen when the access period was extended to 24 hours. In contrast, the Schedule II barbiturate, sodium pentobarbital, produced dose-dependent self-administration at 0.5 and 1.0 mg/kg/infusion (i.v.).

Cumulatively, these two self-administration studies suggest that ramelteon does not produce rewarding effects indicative of abuse liability.

Conditioned Place Preference

Ramelteon (3 - 30 mg/kg, i.p.) did not induce conditioned place preference in rats, similar to the response from melatonin (10 - 100 mg/kg, i.p.) or vehicle. In contrast, known drugs of abuse did produce conditioned place preference, including the Schedule II opioid, morphine (1 mg/kg, s.c.), the Schedule IV benzodiazepine, diazepam (5 mg/kg, p.o.), and the Schedule IV benzodiazepine, triazolam (0.5 mg/kg, p.o.). These data are consistent with the monkey self-administration study showing no significant rewarding properties from ramelteon.

Drug Discrimination

In monkeys trained to discriminate the Schedule IV benzodiazepine, midazolam, ramelteon (3.2 - 10 mg/kg, i.v.) did not generalize to the midazolam interoceptive cue (percent bar pressing on midazolam lever < 20%), similar to vehicle responding. Ramelteon did not affect the rate of responding. These data suggest that ramelteon does not have behavioral effects that are similar to those of benzodiazepine sedative-hypnotics.

Ramelteon (3.2 - 10 mg/kg, i.v.) also did not block the discriminative cue (e.g., signs of withdrawal) produced by the benzodiazepine antagonist, flumazenil (0.0032 - 0.32 mg/kg (cumulative dosing)), in rats that were dependent on the Schedule IV benzodiazepine, diazepam. The inability of ramelteon to reverse the effects of a benzodiazepine antagonist suggests that ramelteon does not act through benzodiazepine receptors. These data are consistent with the midazolam discrimination study.

Drug Interaction Studies

Ramelteon did not affect rotorod performance, an indicator of disruption of motor function, nor did it potentiate the ability of the Schedule IV benzodiazepine, diazepam, to interfere with rotorod performance.

D. Physical Dependence

Ramelteon discontinuation did not induce withdrawal-like behaviors or changes in body weight in monkeys following administration of the drug at 10 mg/kg (i.g.) for one year. Additionally, there were no changes in spontaneous behavior during the discontinuation period, nor were there any changes in conditioned response rates to obtain food or to terminate electric shock.

Similarly, no withdrawal-associated behaviors or changes in body weight were seen following discontinuation of ramelteon (200 or 600 mg/kg, in food) in rats treated with the drug for 28 days. In contrast, discontinuation after 28 days from the Schedule IV benzodiazepine, diazepam (300 mg/kg, in food) produced irritability, a decrease in feeding behavior and a decrease in feces, demonstrating a mild withdrawal syndrome.

Thus, the lack of withdrawal-associated behaviors indicates that ramelteon does not produce physical dependence.

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/s/

Katherine Bonson
6/10/05 11:22:36 AM
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Michael Klein
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