CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205677Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 205677
Submission date: 5/31/2013
Drug: tasimelteon
Applicant: Vanda Pharmaceuticals Inc.
Indication: Treatment of Non-24-hour disorder in the totally blind

Reviewing Division: Division of Neurology Products

Discussion:
The pharmacology/toxicology reviewer and supervisor found the nonclinical information submitted for tasimelteon to be adequate to support approval of this NDA for the indication listed above.

Tasimelteon is a melatonin receptor agonist. This is an existing Established Pharmacologic Class and is acceptable for labeling.

Two-year carcinogenicity studies of tasimelteon were conducted in mice and rats. The executive carcinogenicity assessment committee found the studies to be acceptable. There were no drug-related neoplasms in mice. Liver adenomas and carcinomas were increased in male rats and liver adenomas were increased in female rats. Uterine and cervical squamous cell carcinomas and uterine endometrial adenocarcinomas were also increased in rats. These tumors only occurred at doses that were substantially higher than the human dose on a mg/m^2 basis.

Reproductive and developmental studies in rat and rabbits identified some effects on the dam, fetuses and offspring. These effects are described in the proposed labeling and it is noted that the effects only occurred at doses that were substantially higher than the human dose on a mg/m^2 basis.

Conclusions:
I concur that this application can be approved from the pharmacology/toxicology perspective.

Reference ID: 3441725

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/s/

PAUL C BROWN
01/24/2014

MEMORANDUM

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

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

Date: November 12, 2013

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

Subject: NDA 205-677 (tasimelteon; Hetlioz)

NDA 205-677 was submitted by the sponsor (Vanda Pharmaceuticals Inc.) on May 30, 2013 for tasimelteon as treatment for non-24-hour disorder in the totally bind individual. The application was filed and given a Priority classification (Filing Communication letter, dated 7/29/2013), with a user fee goal date of January 31, 2014. The only nonclinical request was for "…a signed and dated Pathology Report for the pivotal 6-month toxicity study in rats (Study TAJ0007-98348)…," which was submitted to the NDA on August 20, 2013 (SDN 11).

The sponsor conducted a full battery of nonclinical studies in support of clinical development (under IND 54776) and marketing approval. These studies were reviewed in detail by Dr. Melissa K. Banks-Muckenfuss and were found to be adequate to support approval of the NDA, with appropriate labeling (*Pharmacology/Toxicology NDA Review and Evaluation, NDA 205-677, Melissa K. Banks-Muckenfuss, Ph.D., 10/30/2013*).

Comments and Recommendations

Pharmacology: Tasimelteon is a melatonin MT_1 and MT_2 receptor agonist, with high in vitro binding affinity for both receptors, similar to that of melatonin. Three major circulating metabolites were identified in human plasma following oral administration: M9, M12, and M13. In humans, plasma levels of M9 and M12 are higher (1.3- and 1.8-fold, respectively), whereas those of M13 are similar, to those of the parent compound. All three major human metabolites exhibited in vitro binding affinity for the MT_1 and MT_2 receptors. The data (K_i in nM) are summarized in the following table.

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COMPOUND	MT_1	MT_2
tasimelteon	0.30-0.35	0.07-0.17
M9	1180	71.9
M12	136	10.8
M13	4	0.92
melatonin*	0.01-0.33	0.11-0.25

*calculated based on data from Hardeland *et al*. *Expert Opin Investig Drugs* 19(6):747-764, 2010

In proposed labeling, the sponsor has stated [redacted] (b) (4) (b) (4) (b) (4)

The data provided by the sponsor establish tasimelteon as a melatonin MT_1 and MT_2 agonist. The sponsor also conducted three nonclinical studies in Long-Evans hooded rats to assess the "chronobiotic" properties (or adjustment of the timing of internal circadian rhythms) of tasimelteon. In Study 52254, groups of rats were kept for 3-6 weeks either in a room in which the lights-on period was 7 am to 7 pm or in a room in which the lights-on period was 7 pm to 7 am. Two group of rats housed in the 7 pm-to-7 am lights-on room for 3 weeks were transferred to the reverse light-dark cycle for 2 days and then sacrificed. In one of these two groups, animals received three subcutaneous (SC) injections of melatonin (1 mg/kg) or tasimelteon (1 or 5 mg/kg) shortly before transfer (shortly before lights on) and shortly after the beginning of the lights-off period on the next two days; these animals were sacrificed after two days exposure to the reversed light-dark cycle. At sacrifice in all groups, hippocampal slices were prepared and electrical activity in the suprachiasmatic nuclei (SCN) was recorded. The high dose of tasimelteon delayed the peak SCN activity by 1.3 hrs, similar to melatonin; the low dose of tasimelteon had no effect.

In Study 52274, the chronobiotic effects of melatonin and tasimelteon were tested in an animal model of acute phase shifting. Rats were maintained under a 12-hr light/12-hr dark cycle for ~2 weeks, until the lighting conditions and the onset of wheel-running were synchronized. Animals were then placed in continuous darkness for the remainder of the experiment. After two weeks in total darkness, animals received a single SC injection of vehicle, tasimelteon (0.01, 0.1, 1.0, or 5.0 mg/kg), or melatonin (0.1 or 1.0 mg/kg) two hours prior to each animal's anticipated onset of wheel-running. Melatonin advanced the onset of wheel-running at both doses, in a dose-related manner.

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Tasimelteon advanced the onset of wheel-running at 1.0 and 5.0 mg/kg, with a slightly greater effect at 1.0 mg/kg; the lower doses were without effect.

In Study 52273, rats were maintained under light/dark conditions similar to those used in Study 52274. In this experiment, however, after two weeks in total darkness, animals received SC injections of vehicle, melatonin (1.0 mg/kg), or tasimelteon (0.01, 0.1, 1.0, or 5.0 mg/kg) daily for 66 days. The data were expressed as the percentage of animals entrained. Entrainment was considered established when (1) the onset of wheel-running was "locked to injection time" and (2) "free-running" (endogenous) rhythm was reestablished after the end of the dosing period. Melatonin induced entrainment in 100% of treated animals. For tasimelteon, the % of animals entrained was 11.3, 25, 91, and 100% at 0.01, 0.1, 1.0, and 5.0 mg/kg, respectively.

The sponsor considered the results of Studies 52254, 52274, and 52273 as evidence that tasimelteon, like melatonin, exhibits chronobiotic properties. The results obtained with melatonin are consistent with the results of published studies (cf. Arendt J, Skene DJ. *Sleep Med* 9:25-29, 2005). However, the study reports do not provide sufficient data to be considered definitive evidence of a chronobiotic effect for tasimelteon (b) (4)

Toxicology: The pivotal oral toxicity studies of tasimelteon were conducted in Sprague-Dawley rat and cynomolgus monkey, with duration of dosing up to 6 months (0, 5, 50, 500 mg/kg/day) and 1 year (0, 3, 20, 150 mg/kg/day), respectively. The primary target organs were the CNS (convulsions) in both rat and monkey, and liver (centrilobular hypertrophy, focal necrosis), kidney (chronic progressive nephropathy, interstitial macrophages, hyaline droplets and pigment in cortical tubules, cortical tubular dilatation), and spleen (hemosiderosis, extramedullary hematopoiesis) in rat. A dose-related increase in pica was observed in the 6-month rat study (males and females), a behavior thought to reflect a compound's emetogenic potential in this species which cannot vomit (Takeda N *et al*. *Pharm Biochem Behav* 45:817-821, 1993; Yamamoto K *et al. Eur J Pharmacol* 554:34-39, 2007); increased emesis was observed in monkey. These studies provided an adequate assessment of tasimelteon in both species, and the major human metabolites in one or both species. (Plasma exposures for M9, M12, and M13 in the 1-year monkey study were estimated based on data from a single-dose PK study.)

Reproductive and developmental toxicology: Studies were conducted in rat (fertility and early embryonic development [0, 5, 50, 500 mg/kg], embryofetal development [0, 5, 50, 500 mg/kg], and pre/postnatal development [0, 50, 150, 450 mg/kg]) and rabbit (embryofetal development [0, 5, 30, 200 mg/kg]). These studies identified disruption of the estrus cycle (rat), embryolethality, reduced fetal body weight, and delayed fetal development (rabbit), and persistent reductions in pup body weight, delayed physical development, and impaired performance on learning and memory tasks (rat) as tasimelteon-related effects. These findings are consistent with a Pregnancy Category C, as recommended by the sponsor and Dr. Banks-Muckenfuss. It is of note, however, that these findings were primarily observed at the highest doses tested, with the highest no-effect doses generally providing substantial safety margins for humans based on plasma

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levels of parent compound and metabolites M9, M12, and M13 in rat or body surface area (mg/m^2) comparisons for rabbit. (In rabbit, plasma exposure data were not available for parent compound at the highest no-effect dose or for M9, M12, or M13 at any dose.)

Carcinogenicity: Lifetime carcinogenicity studies were conducted in CD-1 mouse (0, 30, 100, 300 mg/kg/day) and Sprague-Dawley rat (0, 20, 100, 250 mg/kg/day). The results of these studies were reviewed by the Division and the Executive CAC (*Executive CAC Meeting Minutes, dated 10/16/2013*). Both studies were considered to be acceptable, and adequately assessed the carcinogenic potential of parent compound (both species) and metabolites M9, M12, and M13 (rat). (Plasma exposure data were not available for M9, M12, or M13 in mouse.) There were no drug-related increases in neoplasms in the mouse study. In rat, the following neoplasms were identified as drug-related: uterus (endometrial adenocarcinoma at the HD), uterus and cervix (squamous cell carcinoma at the HD), and liver (adenoma in MDF and HDF; adenoma and carcinoma combined in MDM and HDM). The positive findings in uterus were statistically significant, whereas those in other organs were identified as drug-related based on the incidences exceeding historical control ranges.

Genetic toxicology: The genotoxic potential of tasimelteon was tested in a standard battery of studies: Ames assay, in vitro cytogenetic assay in primary human lymphocytes, and in vivo micronucleus assay in rat (conducted as part of the 1-month oral toxicity study]). The Ames and the in vivo micronucleus assays were found to be adequately conducted and negative, both with and without metabolic activation. In the in vitro cytogenetics assay, tasimelteon was positive (2-fold increase in % of cells with structural aberrations) in the absence of metabolic activation, at the highest concentration (HC; 200 µg/mL) tested (24-hr exposure); at this concentration, there was a decrease in mitotic index (MI) of approximately 50%. Tasimelteon was also positive at the HC (800 µg/mL) tested (5-hr exposure) in the presence of S9; however, this concentration was excessively cytotoxic (74% decrease in MI). With the 5-hr exposure (+S9), the next lower concentrated tested (400 µg/mL) was associated with a MI similar to control. Dr. Banks-Muckenfuss noted that concentrations between 400 and 800 µg/mL should have been tested under these conditions (5-hr exposure, +S9) and considered the results of the assay to be "equivocal/positive" for clastogenicity. While, Dr. Banks-Muckenfuss' conclusions are reasonable regarding the in vitro cytogenetic assay, the increases in % of cells with structural aberrations were ≤2-fold, even in the presence of maximum recommended or clearly excessive cytotoxicity. Overall, the data suggest a lack of genotoxic potential for tasimelteon.

In addition to the genotoxicity studies for tasimelteon, the sponsor conducted a separate evaluation of the genotoxic potential of metabolite M11, a minor circulating metabolite in humans. M11 was negative in an Ames assay, but was positive in an in vitro cytogenetic assay in CHO cells. In the in vitro cytogenetic assay, M11 was reproducibly positive in the presence, but not in the absence, of metabolic activation at concentrations not consistently associated with excessive cytotoxicity. (The sponsor stated, however, that the high M11 concentrations associated with positive findings exceeded the maximum concentration recommended in ICH guidance [ICH S2(R1), 2012]). The reason for the

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sponsor's concern regarding M11 is unclear. While M11 was undetectable in rat plasma, plasma M11 levels estimated to have been achieved in the mouse carcinogenicity study are 1.1-2.2 times that in humans at the recommended daily dose of 20 mg. Therefore, the carcinogenic potential of this minor human metabolite has been adequately assessed.

Impurities: Twelve process (drug substance) impurities were identified by the sponsor. The specification limits for these impurities are set such the total daily dose of all 12 impurities combined would be (b) (4) Therefore, these impurities are adequately controlled. However, in addition to these impurities, the CMC review team identified two potentially genotoxic impurities (b) (4) which would not be adequately controlled if determined to be genotoxic. The results of an FDA QSAR evaluation resulted in a positive prediction (by all three software programs) for *Salmonella* mutagenicity for (b) (4) but not for (b) (4) An information request was sent to the sponsor (letter dated 9/20/2013) asking for additional information on (b) (4) In submission SDN0020 (10/10/2013), the sponsor proposed a specification limit of NMT (b) (4) for this impurity, and stated that "the total amount of potential genotoxic impurities is NMT (b) (4) If confirmed by the CMC review team, these limits would be acceptable, from a nonclinical standpoint.

Recommendations: The nonclinical studies of tasimelteon submitted by the sponsor support approval of the NDA for the proposed indication, with appropriate labeling. Since tasimelteon has been granted orphan drug status, the sponsor is exempt from the requirement (under PREA) to assess safety and effectiveness in the pediatric population; therefore, no nonclinical postmarketing requirements are recommended.

Additional labeling recommendations will be communicated separately.

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/s/

LOIS M FREED
11/12/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	205-677
Supporting document/s:	NDA 205-677: SDN1; SDN11; SDN16
Applicant's letter date:	NDA 205-677: 05/31/13; 8/20/13; 9/17/13
CDER stamp date:	NDA 205-677: 05/30/13; 8/20/13; 9/17/13
Product:	tasimelteon, proposed tradename Hetlioz (VEC-162, BMS-214778)
Indication:	Treatment of Non-24-Hour Disorder in the Totally Blind
Applicant:	Vanda Pharmaceuticals Inc.
Review Division:	DNP, HFD-120
Reviewer:	Melissa K. Banks-Muckenfuss, Ph.D.
Supervisor/Team Leader:	Lois M. Freed, Ph.D.
Acting Division Director:	Eric P. Bastings, M.D.
Project Manager:	Cathleen Michaloski, BSN, MPH

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of NDA 205-677 are owned by Vanda Pharmaceuticals or are data for which Vanda Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 205-677 that Vanda Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application are for descriptive purposes only and are not relied upon for approval of NDA 205-677.

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Executive Summary

1.1 Introduction

Tasimelteon is a melatonin (MT_1 and MT_2) receptor agonist. The development program has spanned greater than a decade, and the proposed indication has evolved (e.g., insomnia, circadian rhythm disorders, eastward-bound jet lag). The development of the compound was discontinued, with several toxicology studies in progress and terminated early, and was restarted several years later by the current sponsor. The mechanism of action appears similar to marketed drug ramelteon (the EPC will be the same), although the observed toxicities differ slightly. On 1/19/10, the sponsor was granted an orphan drug designation for tasimelteon for the treatment of Non-24-hour Sleep-Wake Disorder in Blind Individuals With No Light Perception.

1.2 Brief Discussion of Nonclinical Findings

Tasimelteon showed affinity at melatonin (MT_1 and/or MT_2) receptors, with slightly greater affinity at MT_2 receptors; tasimelteon's metabolites showed at least a 10-fold lower affinity at melatonin receptors. In an early *in vitro* receptor binding study, tasimelteon was shown to have affinity similar to melatonin at MT_1 receptors and greater than melatonin at MT_2 receptors. Tasimelteon did not show affinity at other targets in a receptor binding screen.

The primary target organs of tasimelteon toxicity include the: CNS, liver, kidney, and reproductive organs. Some alterations were also observed in spleen and heart, as well as the hematologic and endocrine systems (i.e., thyroid, pituitary, and adrenal glands). Generally, slight reductions in weight and sometimes food consumption were observed across the studies. CNS signs including convulsions (clonic and/or tonic), tremors, loss of righting reflex, flaccidity, ataxia, and/or labored breathing were observed at high doses in studies in rats, mice, and monkeys. Liver was clearly a target organ in most of the toxicological species used; increased liver enzymes (as well as cholesterol and/or triglycerides), increased liver weight and size, histopathological alterations (i.e., centrilobular to panlobular hepatocellular hypertrophy, increased hepatocellular mitoses, bile duct hyperplasia, cystic degeneration, hepatocyte vacuolation, regenerative hyperplasia, centrilobular fatty change, focal pigmented macrophages, pigment in hepatocytes, focal hepatocyte necrosis, and/or periportal/serosal/subserosal fibrosis), and tumors were observed. Kidney toxicity was observed in rats (i.e., increased kidney weight; exacerbated CPN; minimal to slight cortical tubules with hyaline droplets; minimal to moderate cortical tubular pigment; interstitial pigmented macrophages; slight cortical tubular dilation; pelvic dilatation; interstitial inflammation; and/or simple tubular hyperplasia), with minimal evidence in mice and monkeys. Changes in reproductive organs were observed in rats (e.g., decreased prostate weight; testicular interstitial cell hyperplasia; altered estrus cycling; increased ovary weight; ovarian follicular cysts; uterine dilatation; uterine adenocarcinoma; uterine squamous cell carcinoma; uterine cervix epithelial hyperplasia and/or keratinization; and uterine cervix squamous cell carcinoma), mice (e.g., decreased prostate weight) and monkeys (e.g., decreased prostate, seminal vesicle, and testes weights; lack of corpora lutea in the ovary). Slight

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reductions (5-15%) in red blood cell parameters (and slight increases in platelets) were observed in rats and monkeys. In rat spleen, hemosiderosis and extramedullary hematopoiesis were observed; the sponsor believed these findings reflected an increased rate of RBC removal from peripheral blood leading to a "compensatory" response. A few effects were observed among the studies in the thyroid (e.g., increased weight; follicular cysts, atrophy, and/or hypertrophy) and pituitary (e.g., focal hyperplasia). The no adverse effect levels for the general toxicity studies were within the clinical range; however, effect doses were approximately 20 times the recommended human dose (RHD) of 20 mg on a mg/m^2 basis.

Tasimelteon was negative in an in vitro Ames assay and an *in vivo* rat micronucleus assay; however, an *in vitro* chromosomal aberration assay demonstrated clastogenic effects. Tasimelteon metabolite M11 (a non-major human circulating metabolite that has poor coverage in the animal species) was also negative in an in vitro Ames assay but was clastogenic in an *in vitro* chromosomal aberration assay in CHO cells. Two year carcinogenicity bioassays were conducted in rats and mice. In rats, tumors were observed in the liver (≥ MD; adenomas in F and adenomas and carcinomas in males), uterus (endometrial adenocarcinomas, HDF), and uterus and cervix (squamous cell carcinomas, HDF). The relevance of these tumors to humans is unknown. Tumors were not observed at approximately 10 times the RHD, on a mg/m^2 basis. No drug-related neoplasms were reported in mice.

As suggested in the toxicity studies (e.g., effects on reproductive organs, such as a slightly increased incidence of absent corpora lutea in the rat carcinogenicity study and the lack of corpora lutea in the ovaries of female monkeys in the chronic toxicity study), effects on female rat fertility and fetal development in rats and rabbits (into adulthood in rats) were demonstrated in the reproductive toxicology studies. Irregular estrus cycles, slightly increased infertile pairings, and slight reductions in fertility parameters were observed at MD and HD in females; the no-effect dose was approximately 2.5 the RHD on a mg/m^2 basis. The embryofetal development (EFD) studies in rats and rabbits demonstrated some embryofetal toxicity. Drug-related slight delays in development and decreased fetal body weights were observed at MD and HD in rats and rabbits; rabbits also demonstrated an increase in abortions and slightly increased post-implantation losses (late resorptions) at MD and HD. Maternal toxicity was also observed (i.e., clinical signs, and slight reductions in body weight and food consumption) in the dams and does at HD. The no-adverse-effect doses in rats and rabbits were approximately 25 and 30 times the RHD on a mg/m^2 basis. In the pre/postnatal development study (dosing the dams throughout gestation and lactation), clear toxicity in the F1 generation was demonstrated as developmental delays and a reduced growth rate postpartum and into adulthood. The gestation period was very slightly increased in all VEC-162-treated dams, and the live birth index was slightly reduced at HD. Although average pup birth weights were similar (<5% difference; provided as the average of the litter averages for each sex), HD pups demonstrated reduced (~15%) body weights throughout lactation and into adulthood. Sexual maturation was achieved at a similar age (PND) but at a lower body weight in males, but was delayed in females. Other developmental milestones (e.g., righting responses) were similarly delayed. Neurobehavioral testing

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(i.e., Morris Water maze) demonstrated clear deficits in males (i.e., trial times, sector entries, and failed trials) but not females, when tested at PND 31/32; when re-tested at PND 47/49, no consistent differences were reported. A few effects on F_1 generation reproduction were observed. Reductions in body weight continued in the adult female F_1 generation during gestation, and slight effects on fertility were observed at HD. The F_1 generation was exposed to VEC-162, M9, M12 and M14 (as measured on LD4), although to a much lesser extent than the dams. Limited F_0 maternal toxicity was demonstrated (i.e., slightly [~3-5%] reduced body weights during gestation at MD and/or HD, and during lactation in all VEC-162-treated dams). The no-effect dose was approximately 2.5 times the RHD on a mg/m^2 basis.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, the application is recommended for approval.

While most of the observed toxicities occurred at exposures exceeding those anticipated at the RHD or would be monitorable in humans (e.g., liver enzyme increases), the carcinogenic and reproductive effects are considerable. Taking into account the orphan population and toxicities identified for similar drugs (e.g., tumors and malformations observed with ramelteon), the identified toxicities do not preclude approval but should be fully disclosed in the labeling. Of particular importance are: the tumors in the liver, uterus, and uterine cervix, the altered cyclicity observed in female rats and suggested in female monkeys (and potential effects on fertility in humans), and the persistent effects on growth of offspring exposed during gestation and lactation (i.e., exposed *in utero* in humans).

1.3.2 Additional Non Clinical Recommendations

Please see suggested labeling.

1.3.3 Suggested Labeling

Final labeling requires discussion among the review team members, as well as with the sponsor. This suggested labeling is provided in case the application moves to approval, and represents the reviewer's current thoughts, but does not reflect final label wording.

Highlights:

INDICATIONS AND USAGE

Hetlioz is a melatonin receptor agonist indicated for the treatment of Non-24-Hour Disorder (Non-24) in the totally blind. (1)

USE IN SPECIFIC POPULATIONS

- Pregnancy: Based on animal data, may cause fetal harm. (8.1)

1. Indications and Usage

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Hetlioz is a melatonin receptor agonist indicated for the treatment of Non-24-Hour Disorder (Non-24) in the totally blind.

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of Hetlioz in pregnant women. In animals, (b) (4) Hetlioz should be used during pregnancy only if the potential benefit justifies the potential risks.

In pregnant rats administered tasimelteon at oral doses of 5, 50, or 500 mg/kg/day during the period organogenesis, (b) (4) (b) (4) and food consumption. The (b) (4) was approximately 30 times the RHD on a mg/m^2 basis.

Oral administration of tasimelteon (50, 150, or 450 mg/kg/day) to rats throughout (b) (4) and lactation resulted in (b) (4) (b) (4) (b) (4) The no-effect dose for offspring was approximately (b) (4) times the RHD on a mg/m2 basis.

8.3 Nursing mothers

It is not known whether Hetlioz is secreted into human milk, (b) (4) Because many drugs are excreted into human milk, (b) (4)

12.1 Mechanism of action

(b) (4)

The mechanism by which tasimelteon exerts its therapeutic effect in Non-24 Hour Disorder is unknown. Tasimelteon's (b) (4) at MT_1 and MT_2 receptors (b) (4) are thought to be involved in the (b) (4) e of the circadian rhythm (b) (4)

13.1 Carcinogenesis, mutagenesis, impairment of fertility

Carcinogenesis

(b) (4)

Mutagenesis

(b) (4)

Impairment of Fertility

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number 609799-22-6

CAS Name Propanamide, N-[[1R,2R)-2-(2,3-dihydro-4-benzofuranyl)cyclopropyl]methyl]-

Generic Name tasimelteon (INN, USAN)

Code Name VEC-162 (Vanda), BMS-214778 (BMS), (b) (4) (b) (4)

Chemical Name (1*R-trans*)-*N*-[[2-(2,3-Dihydro-4-benzofuranyl)cyclopropyl]methyl]propanamide

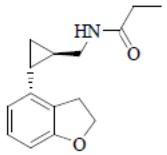
N-[[(1*R*,2*R*)-2-(2,3-Dihydro-1-benzofuran-4-yl)cyclopropyl]methyl]propanamide

(1*R*, 2*R*)-*N*-[2-(2,3-Dihydrobenzofuran-4-yl)cyclopropylmethyl]propanamide

propionamide derivative of 4-(2-methylcyclo-propyl)-2,3-dihydrobenzofuran

Molecular Formula/Molecular Weight $C_{15}H_{19}NO_2$; 245.32

Structure or Biochemical Description



(b) (4)

Pharmacologic Class melatonin receptor agonist

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2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 54,776, the associated IND for the treatment of circadian sleep disorders
IND 112, 702, MDD (DPP)

2.3 Drug Formulation

See sponsor's Table 3.2.P.1-2, below.

Table 3.2.P.1-2: Composition of Tasimelteon 20-mg Capsules

Component	Reference to standard	Weight (mg) capsule	% w/w composition per capsule
Tasimelteon drug substance	Vanda (Section 3.2.S.4.1)	20.00[1]	(b) (4)
Lactose anhydrous	NF		(b) (4)
Microcrystalline cellulose (b) (4)	NF/EP/JP		
Colloidal silicon dioxide (b) (4)	NF/EP/JP		
Croscarmellose sodium	NF/EP/JP		
Magnesium stearate (b) (4)	NF/EP/JP		
(b) (4)			
Size 1, dark blue opaque, hard gelatin capsules printed with "VANDA 20 mg" in white[2]			(b) (4)
Total capsule weight		376.00	

[1] Weight is adjusted for % assay of drug substance.
[2] Capsules used for stability and clinical batches were printed with (b) (4) but validation and commercial batches will have "VANDA 20 mg" printed in white.
(b) (4)

2.4 Comments on Novel Excipients

In section 3.2.P.4, the sponsor stated that there are no novel excipients; all excipients used in the manufacture of tasimelteon 20-mg capsules are commonly used USP and/or NF compendial excipients.

2.5 Comments on Impurities/Degradants of Concern

The sponsor identified a number of potential organic impurities. The sponsor provided Table 3.2.S.3.2-1 to describe 12 synthetic impurities (see below); of these, (b) (4) were identified as potentially genotoxic compounds by the sponsor. The sponsor stated that these compounds are controlled at a total combined specification of (b) (4) levels, which does not exceed (b) (4) at the clinical dose of 20 mg (see sponsor's Table 2.3.S.4-1). The CMC

reviewer (see review by Dr. Kambhampati) indicated that all potential genotoxins but two (b) (4) were adequately controlled. Those two compounds were referred to FDA's Computational Toxicology group, which identified (b) (4) as a potential genotoxin. The CMC reviewer has requested additional information from the sponsor.

(b) (4)

(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

Hetlioz (20 mg tasimelteon capsules) is proposed for the treatment of Non-24-Hour Disorder in the totally blind, a circadian rhythm disorder that occurs when individuals are "unable to entrain (synchronize) their endogenous master body clock to the 24-hour day-night cycle." Tasimelteon was granted an orphan drug designation, for the treatment of "Non-24-Hour Sleep-Wake Disorder in Blind Individuals with No Light Perception."

2.7 Regulatory Background

The sponsor has previously proposed tasimelteon for the following indications: the treatment of Insomnia, Circadian Rhythm Sleep Disorders, and Jet Lag Disorder due to eastward travel. While being developed by BMS, tasimelteon (then known as BMA-214778) development was discontinued; the ongoing nonclinical studies were terminated early (e.g., the 6-month rat toxicity study, the 12-month monkey toxicity study, the fertility and early embryonic development study). Vanda Pharmaceuticals purchased the development program and again began to develop tasimelteon. The sponsor hired CROs to complete and finalize the nonclinical studies that had been terminated early, which is unusual; however, the studies have the GLP and QA statements (note that the report of the 6-month rat toxicity study did not contain a separate, signed pathology report; however, it was provided upon request).

3 Studies Submitted

3.1 Studies Reviewed

Overview					Test Article: tasimelteon	
Species and Strain	**Method of Administration**	**Duration of Dosing**	**Doses (mg/kg)[a]**	**GLP Compliance**	**Testing Facility**	**Study Number**
Repeat-dose toxicity						
Rat/Hsd:Sprague-Dawley®SD®	Oral gavage	26 weeks	0, 5[a], 50, 500	Yes	BMS, Mt. Vernon, IN and Princeton, NJ, (b) (b) (4)	TAJ0007 (98348)
Cynomolgus Monkey	Nasogastric intubation (or oral gavage when necessary)	1 year	0, 3, 20, 150[c]	Yes	(b) (4)	057-005 (98353)
Genotoxicity *in vitro*						
Primary human lymphocytes	NA	NA	0 to 200 µg/ml (-S9) and 0 to 800 µg/ml (+ S9); 4 dose levels per assay	Yes	BMS, Syracuse, NY	97680
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2 uvra	NA	NA	0 to 5,000 µg/plate (5 to 6 dose levels per assay)	Yes	(b) (4)	UNN0003[d]
Chinese Hamster Ovary Cells	NA	NA	0 to 5,000 µg/ml (5 to 8 dose levels per assay)	Yes		UNN0004[d]
Carcinogenicity						
Mouse/Crl: CD-1™ (ICR) BR	Oral gavage	104 weeks	0, 30, 100[a], 300	Yes		TAJ0002
Rat/Crl:CD® (SD)IGS BR	Oral gavage	104 weeks	0, 20[a], 100, 250	Yes		TAJ0001
Reproductive and developmental toxicity						
Fertility and early embryonic development						
Rat/Crl:CD® (SD) IGS BR	Oral gavage	Females: 2 weeks before pairing until 7 days after mating; males: 4 weeks before first pairing and throughout pairing until termination	0, 5, 50[e], 500[f]	Yes	BMS, New Brunswick, NJ.	TAJ0008 (99009)
Embryo-fetal development						
Rat/Crl:CD® (SD) IGS BR	Oral gavage	10 days (Days 6 through 15 of gestation)	0, 5, 50, 500[g]	Yes	BMS, New Brunswick, NJ	99005
Rabbit/ Hra:(NZW)SPF	Oral gavage	13 days (Days 7 through 19 of gestation)	0, 5, 30, 200[h]	Yes	BMS, New Brunswick, NJ	99001

Prenatal and postnatal development, including maternal function					(b) (4)	
Rat/Crl:CD (SD)	Oral gavage	From Day 6 after mating to Day 10 of lactation	0, 50, 150, 450	No		TAJ0015
Rat/Crl:CD (SD)	Oral gavage	From Day 6 after mating to Day 20 of lactation	0, 50[a], 150, 450	Yes		TAJ0017
Local Tolerance						
Bovine Cornea	NA	NA	0, 20%	Yes		99AB38.350
Guinea Pig/ Dunkin/Hartley	Intradermal (I.d) injection and topical application	I.d and topical induction, topical challenge after 2 weeks, 2nd challenge 1 week later.	Induction: 0, 2.5% (0.1 ml i.d.), 0, 50% (0.4 ml topical); 1st challenge:15%, 30% (0.2 ml topical);2nd challenge 7.5%, 15% (0.2 ml topical);	Yes		BMY 378/993273/SS
Other Studies						
Photosafety (Molar Extinction Coefficient Determination)						
NA	NA	NA	NA	No		VCR-TMI-121012

[a] The highest No Observed Adverse Effect Level (NOAEL) is underlined, the No Observed Effect Level (NOEL) is double underlined.
[b] The highest dose of 800 mg/kg was lowered to 600 mg/kg on Study Day 2 because it was poorly tolerated after one dose and resulted in the early mortality of eleven mice.
[c] NOAEL was not conclusively established.
[d] This study was conducted with tasimelteon metabolite M11.
[e] NOAEL for systemic toxicity and female fertility.
[f] NOAEL for male fertility and early embryonic development.
[g] The NOEL was not specifically noted in the report; however, there were no compound related malformations and tasimelteon did not cause selective embryo-fetal toxicity at any dose.
[h] NOEL was not specifically noted in the report; however, there were no compound related malformations and tasimelteon did not cause selective embryo-fetal toxicity at any dose.
BMS= Bristol-Myers Squibb Company; (b) (4)
(b) (4) NA= Not Applicable; i.d. = Intradermal; + S9 = in the presence of S-9 mix; - S9 = in the absence of S-9 mix.

Numerous pharmacology and pharmacokinetics studies were briefly or fully reviewed, including those listed below (not exhaustive).

APPEARS THIS WAY ON ORIGINAL

Overview		**Test Article:** tasimelteon and its main metabolites		
Type of Study	**Test System**	**Method of Admin.**	**Testing Facility**	**Study Number**
Primary Pharmacodynamics				
Affinity and efficacy of tasimelteon at the human melatonin receptors	NIH-3T3 Cells	*In Vitro*	BMS Pharmaceutical Research Institute, Wallingford, CT	52253
Assessment of the interaction of tasimelteon with a comprehensive panel of receptors, binding sites and enzymes	Radioligand binding and enzyme assays	*In Vitro*	(b) (4)	52186
Extended receptor binding profile of tasimelteon	Radioligand Binding Assays	*In Vitro*	(b) (4) (b) (4)	1095880
Affinity of tasimelteon and metabolites M9, M11, M13, M12 & M14 at the human melatonin MT_1 and MT_2 receptors	Radioligand Binding Assays	*In Vitro*	(b) (4) (b) (4)	AA82606
Further characterization of the affinity and efficacy of tasimelteon and metabolites M9, M11, and M13 at the human melatonin MT_1 and MT_2 receptors	Radioligand Binding Assays	*In Vitro*	(b) (4) (b) (4)	AA85237
Further characterization of the affinity and efficacy of tasimelteon and metabolites M12 and M14 at the human melatonin MT_1 and MT_2 receptors	Radioligand Binding Assays	*In Vitro*	(b) (4) (b) (4)	AB04244
Affinity and efficacy of metabolite M3 at the human melatonin MT_1 and MT_2 receptors	Radioligand Binding Assays	*In Vitro*	(b) (4) (b) (4)	AB13313
Extended receptor binding profile of metabolites M9, M11, and M13	Radioligand Binding Assays	*In Vitro*	(b) (4) (b) (4)	AA98075
Extended receptor binding profile of metabolites M12 and M14	Radioligand Binding Assays	*In Vitro*	(b) (4) (b) (4)	AB04243
Extended receptor binding profile of metabolite M3	Radioligand Binding Assays	*In Vitro*	(b) (4) (b) (4)	AB13314
Tasimelteon activity in an *ex vivo* model of chronobiotic modulation	Rat	s.c.	BMS Pharmaceutical Research Institute, Wallingford, CT	52254
Tasimelteon ability to shift the onset of running-wheel activity	Rat	s.c.	BMS Pharmaceutical Research Institute, Wallingford, CT	52274
Tasimelteon ability to entrain running-wheel activity	Rat	s.c.	BMS Pharmaceutical Research Institute, Wallingford, CT	52273
Safety Pharmacology				
Effects of tasimelteon on cloned hERG potassium channels expressed in human embryonic kidney cells	Human Embryonic Kidney Cells	*In Vitro*	(b) (4)	070227.WBO
Effects of tasimelteon on action potentials in isolated rabbit cardiac Purkinje fibers	Rabbit cardiac Purkinje fibers	*In Vitro*		070228.WBO
Distribution				
Mass balance following single oral or i.v. administration and tissue distribution following single oral administration of [^{14}C]-tasimelteon in rats*	Rat	Oral and i.v.	(b) (4) (b) (4)	178/214778/003
In vitro serum protein-binding of [^{14}C]-tasimelteon in human, monkey, rat and mouse	*In vitro*	NA	BMS-PRI	910073823

			(b) (4)	
Metabolism				
Single-dose study with i.v. administration of tasimelteon in rats	Rat	i.v.		8267772
Single-dose study with oral administration of [^{14}C]-tasimelteon in rats[a]	Rat	Oral		PHZ00011
Metabolite radio-profiling and identification after single oral dose of [^{14}C]-tasimelteon to rats[ratsb]	*In vitro*	NA		XBL07816
Single-dose study with oral administration of [^{14}C]-tasimelteon in monkeys[a]	Monkey	Oral		PHZ00012
Metabolite radio-profiling and identification after single oral dose of [^{14}C]-tasimelteon to monkeys[monkeysc]	*In vitro*	NA		XBL07815
Repeated-oral dose pharmacokinetic study with tasimelteon in rats[a]	Rat	Oral		TAJ0014
Repeated-dose pharmacokinetic study with tasimelteon in pregnant rabbits	Rabbit	Oral		TAJ0016
Repeated-oral dose pharmacokinetic study with tasimelteon in mice[a]	Mouse	Oral		TAJ0018
Determination of tasimelteon metabolite M11 in mouse plasma	Mouse	Oral		XBL09628
Excretion				
Mass balance following single oral or i.v. administration and tissue distribution following single oral administration of [^{14}C]-tasimelteon in rats[a]	Rat	Oral and i.v.		178/214778/003
Pharmacokinetic Drug Interactions				
Evaluation of tasimelteon as an inducer of liver microsomal cytochrome P450 and UDP glucuronosyltransferase expression	Rat, *ex vivo*	Oral		XT062005

[a] This study was conducted in compliance with Good Laboratory Practice regulations.
[ratsb] Bioanalysis portion of Study PHZ00011.
[monkeysc] Bioanalysis portion of Study PHZ00012.
BMS-PRI= Bristol-Myers Squibb Pharmaceutical Research Institute; (b) (4) LHPLC= High-performance liquid chromatography–mass spectrometry/mass spectrometry; UPLC-MS/MS= ultra-performance liquid chromatography–mass spectrometry/mass spectrometry; NA=Not applicable; NR = Not reported; i.v. = intravenous; UDP Glucuronosyltransferase = Uridine 5'-diphospho-glucuronosyltransferase; (b) (4) (b) (4)

3.2 Studies Not Reviewed

Studies previously reviewed are not reviewed in full here; a few studies previously reviewed are briefly described.

3.3 Previous Reviews Referenced

Dr. A. Atrakchi review, dated 3/7/00
ExecCAC Meeting Minutes, dated 8/23/99
Dr. A. Atrakchi review, dated 2/19/98
Dr. A. Atrakchi review, dated 1/16/98
ExecCAC Meeting Minutes, dated 10/16/13

4 Pharmacology

4.1 Primary Pharmacology

Tasimelteon (also known as BMS-214778 and VEC-162) and its metabolites were evaluated in a number of receptor binding screens. Tasimelteon was shown to be a full agonist at melatonin receptors MT_1 and MT_2 (IC_{50}s of 0.35 nM and 0.17 nM, respectively, and pKis of 9.5 and 9.8, respectively). These values were similar to melatonin at MT_1 and greater than melatonin at MT_2. Tasimelteon also demonstrated potent, concentration-dependent inhibition of forskolin-stimulated cAMP accumulation (EC_{50} at MT_1= 0.74 nM; EC_{50} at MT_2= 1 nM). Later studies with tasimelteon and metabolite M13 showed nanomolar affinity at human melatonin receptor MT_1; tasimelteon and metabolites M11, M12, M13, and M14 showed nanomolar affinity at human melatonin receptor MT_2, although the affinity of tasimelteon was considerably greater than those of the metabolites (see excerpt from the sponsor's submission, below).

PRIMARY BIOCHEMICAL ASSAY								
	COMPOUND	PT NUMBER	SPECIES	CONC.	% INH.	IC_{50} *	K_i	n_H
251600	Melatonin MT_1							
	VEC-162	1114595	hum	1 nM	57	0.586 ± 0.025 nM	0.304 ± 0.013 nM	0.727 ± 0.021
	M13	1114594	hum	10 nM	52	7.69 ± 0.416 nM	4 ± 0.216 nM	0.756 ± 0.006
	M11	1114592	hum	1 µM	62	0.481 ± 0.047 µM	0.250 ± 0.024 µM	0.881 ± 0.060
	M9	1114591	hum	3 µM	50	2.26 ± 0.346 µM	1.18 ± 0.179 µM	0.758 ± 0.011
251700	Melatonin MT_2							
	VEC-162	1114595	hum	0.3 nM	60	0.133 ± 0.014 nM	0.0692 ± 0.007 nM	0.846 ± 0.041
	M13	1114594	hum	3 nM	54	1.78 ± 0.430 nM	0.922 ± 0.224 nM	0.787 ± 0.029
	M11	1114592	hum	10 nM	55	6.63 ± 1.28 nM	3.44 ± 0.663 nM	0.772 ± 0.061
	M9	1114591	hum	0.3 µM	61	0.139 ± 0.005 µM	0.0719 ± 0.003 µM	0.655 ± 0.026

PRIMARY BIOCHEMICAL ASSAY								
	COMPOUND	PT NUMBER	SPECIES	CONC.	% INH.	IC_{50} *	K_i	n_H
251600	Melatonin MT_1							
	M14	1150293	hum	1 µM	77	0.198 µM	0.103 µM	0.801
	M12	1150294	hum	1 µM	70	0.261 µM	0.136 µM	0.688
251700	Melatonin MT_2							
	M14	1150293	hum	10 nM	53	8.42 nM	4.37 nM	0.659
	M12	1150294	hum	0.1 µM	74	0.0208 µM	0.0108 µM	0.696

Metabolite M3 showed low affinity at human melatonin receptors MT_1 and MT_2 (see excerpt from the sponsor's submission below).

Cat #	Assay Name	Species	Conc.	% Inh.	IC_{50}*	K_I	n_H
251700	Melatonin MT_2	hum	1 µM	61	0.36 µM	0.18 µM	0.48
251600	Melatonin MT_1	hum	10 µM	52	3.37 µM	1.75 µM	0.48

Reference ID: 3399188

In the clinical pharmacology summary, the sponsor provided the following summary table of binding affinities at melatonin receptors for tasimelteon, and metabolites M3, M9, M11, M12, M13, and M14. There appeared to be an error in the Ki for M12 at MT_2 (corrected below by the reviewer).

Table 1: Comparative affinity of tasimelteon and its metabolites for the human melatonin MT_1 and MT_2 receptors

Compound	MT_1		MT_2		Ratio K_i MT_1/ K_i MT_2
	IC_{50} (nM)	K_i (nM)	IC_{50} (nM)	K_i (nM)	
Tasimelteon	0.586	0.304 0.350[a]	0.133	0.0692 0.170	4.4 2.1
M3	3,370	1,750	360	180	9.7
M9	2,260	1,180	139	71.9	16.4
M11	481	250	6.63	3.44	72.7
M12	261	136	20.8	~~108~~ 10.8	1.3
M13	7.69	4	1.78	0.922	4.3
M14	198	103	8.42	4.37	23.6

[a] Source Data for this value: Study 52253; all other data : Study AA85237 (tasimelteon, M9, M11, M13), Study AB04244 (M12 and M14), and Study AB13313 (M3), which used identical melatonin receptor assays and the same IC_{50} and Ki calculation methods.

IC_{50} = Concentration producing 50% inhibition; Ki= dissociation constant for the inhibitor.

The role of tasimelteon (BMS-214778) as a "chronobiotoic" was investigated in one *ex vivo* and two *in vivo* studies. In the *ex vivo study*, suprachiasmatic nucleus (SCN) electrical activity was recorded from brain slices taken from rats administered melatonin (1 mg/kg) or tasimelteon (1 and 5 mg/kg) the day prior to and for 2 days following a reversed light-dark cycle (i.e., light onset delayed by 12 hr); SCN electrical activity rhythms shifted significantly faster in the slices taken from melatonin- tasimelteon-treated animals than in slices from the vehicle-treated animals. In an *in vivo* study, tasimelteon (1.0 and 5.0 mg/kg SC) and melatonin (0.1 and 1.0 mg/kg SC) advanced the onset of running-wheel activity in rats housed in constant darkness in a model of acute phase shifting of locomotor activity in rats. In a second *in vivo* study, tasimelteon (1.0 and 5.0 mg/kg SC; 66 daily injections) and melatonin (1.0 mg/kg SC) were reported to "synchronize" the "free-running" locomotor rhythms so that the onset of activity coincided with the injection time in a model of entrainment of "free-running" activity rhythms in rats. All rats treated with 5.0 mg/kg tasimelteon and 90% of those treated with 1.0 mg/kg entrained to the injection time; the ED_{50} for the tasimelteon entrainment effect was 0.21 mg/kg.

Reference ID: 3399188

4.2 Secondary Pharmacology

Tasimelteon and its metabolites were evaluated in a number of receptor binding screens. At concentrations up to 10 µM, no other binding was observed. MT_3 receptor binding was not assessed.

4.3 Safety Pharmacology

The sponsor did not conduct the standard battery of safety pharmacology studies. CNS and respiratory safety pharmacology studies were not conducted, and the cardiovascular safety pharmacology study in dogs was conducted pre-2000 and not under GLP. A few other cardiovascular safety pharmacology studies were conducted, including GLP hERG and action potential duration assays.

The hemodynamic effects of tasimelteon were investigated in anesthetized and conscious dogs after IV administration (non-GLP study). In anesthetized dogs, tasimelteon induced a 14% reduction in mean arterial blood pressure (MABP) at 3.0 mg/kg, with a steady reduction (maximum 17%) in heart rate (HR) over the dose range tested (0.1 – 3.0 mg/kg). In conscious dogs, no changes in MABP, HR, or ECG (including QT) were reported to result from tasimelteon administration. Tasimelteon (maximum concentration tested 100 µM) inhibited hERG current by 14.0 ± 2.2% at 100 µM; an IC_{50} was not calculated. In an *in vitro* isolated rabbit cardiac Purkinje fiber action potential duration assay, tasimelteon produced statistically significant shortening of the APD_{90} after exposure to 100 µM tasimelteon at 1 and 0.5 second basic cycle lengths; no statistically significant changes in resting membrane potential, action potential amplitude, or the maximum rate of depolarization were reported. The maximum rate of depolarization was reduced slightly, but this difference did not reach statistical significance.

In vitro non-GLP studies in rat anterior cerebral artery (ACA) and isolated rat caudal artery (CA) showed that, with lower potency than melatonin, tasimelteon was vasoconstrictive in pressurized (60 mm Hg, constant) ACA and CA in a concentration-dependent manner (EC_{50} estimated to be 500 nM and 180 nM, respectively).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Many PK/ADME studies were conducted by the sponsor during the development of BMS-214778/VEC-162/tasimelteon. Where newer studies were available to provide data, the older studies were not reviewed since the understanding of tasimelteon's ADME has evolved extensively over the last 5 years.

Analytical

A GLP validation of an LC-MS/MS method for the measurement of tasimelteon in the plasma of rats (0.2- 100 ng/mL in a volume of 200 µL; study TAJ0005) and mice (2-1000 ng/mL in a volume of 100 µL; TAJ0004) was conducted. Only an older (1999)

Reference ID: 3399188

validation of an LC-MS/MS method for the measurement of BMS-214778 in the plasma of monkeys (2- 1000 ng/mL in a volume of 15 µL; 910074952) was provided.

GLP validation of an LC-MS/MS method for the measurement of tasimelteon, M9, M12, M13 (all 5- 5000 ng/mL in a volume of 25 µL), and M14 (all 5.438- 5438 ng/mL in a volume of 25 µL; 208-1104) in rat plasma was conducted.

GLP validation of an LC-MS/MS method for the measurement of metabolite M3 (5- 5000 ng/mL in a volume of 25 µL; 208-1213) in rat plasma was conducted.

A partial (from human plasma) GLP validation of an LC-MS/MS method for the measurement of tasimelteon and M11 in rabbit plasma (0.3- 300 ng/mL in a volume of 100 µL; study 208-1103) was conducted. Two partial (from human plasma) GLP validations of an LC-MS/MS and a UPLC method for the measurement of tasimelteon (5- 5000 ng/mL in a volume of 25 µL) and metabolite M11 in mouse plasma (1- 1000 ng/mL in a volume of 25 µL; 208-1204 and 208-1214) was conducted.

ADME

Generally, orally administered tasimelteon is rapidly absorbed, widely distributed, and has a relatively short half-life. Following oral administration of [^{14}C]-tasimelteon (BMS-214778) to rats, plasma levels of radioactivity declines through 120 hr and were not detected by 168 hr post-dose. The tissues with the highest mean concentrations (in order) were stomach, small intestine, liver, kidney, plasma, large intestine, blood, and adrenals; the only tissues still showing radioactivity after 168 hr were eyes, liver, thyroid, lungs, and kidneys. Based on the long half-life of radioactivity in the eyes (126 hr) and longer half-life in pigmented versus non-pigmented skin (15.4 vs. 7.6 hr) following dosing of [^{14}C]-tasimelteon (BMS-214778), binding of tasimelteon to melanin was suggested. Terminal half-life of the radioactivity was also extended in lung (66 hr), kidneys (49 hr), liver (47 hr), adrenal (40 hr),and stomach (22 hr). The half-life of elimination of the radioactivity from brain was approximately one hour. Moderate, concentration-dependent serum protein binding was observed in human, monkey, rat, and mouse. See the sponsor's summary table, below.

Total Conc (ng/mL)	Mean (SD) % Bound in Human	Total Conc (ng/mL)	Mean (SD) % Percent Bound in		
			Monkey	Rat	Mouse
10	90.3 (0.50)	200	79.7 (0.22)	84.8 (0.12)	77.6 (0.19)
50	89.9 (0.26)	1,000	78.2 (0.23)	83.5 (0.14)	77.5 (0.64)
200	89.1 (0.46)	5,000	74.0 (0.49)	80.6 (0.25)	76.3 (0.08)
1,000	87.7 (0.19)	10,000	71.9 (0.51)	78.0 (0.97)	75.3 (0.31)
2,000	85.8 (0.27)	20,000	69.0 (0.75)	76.8 (0.58)	73.5 (0.31)

Reference ID: 3399188

Tasimelteon was shown to induce liver enzymes in rats (STUDY (b) (4) 062005- Amended report; liver tissue taken from rats in the TK arm of the carcinogenicity bioassay); after administration of 100 and 250 mg/kg tasimelteon for 26 weeks, dose-dependent increases in cytochrome b_5, cytochrome P450, and NADPH cytochrome c reductase activities were observed in male and female rats. Although only NADPH-cytochrome c reductase activity was increased more than 2-fold, a 30-40% induction was observed for the cytochrome b_5 and cytochrome P450 enzymes. Tasimelteon was identified as a moderate phenobarbital-type inducer of CYP2B1/2 (17-36% as effective as phenobarbital in 7-pentoxyresorufin O-dealkylation and 70-100% as effective as phenobarbital in testosterone 16b-hydroxylation), a moderate isoniazid-type inducer (70-80% as effective as isoniazid at inducing CYP2E1), and a mild dexamethasone-type inducer (20-70% as effective as dexamethasone at inducing CYP3A1/2). In addition to Phase I enzymes, tasimelteon was shown to induce Phase II UGT enzymes (i.e., UGT1A6/7, UGT1A1/6, and UGT2B2). See excerpt from the sponsor's report regarding the demonstrated effects on CYP P450 enzymes, following.

> Dosing of rats with tasimelteon caused dose-dependent increases in 7-pentoxyresorufin *O*-dealkylation (CYP2B1/2) in both male and female rats (up to 2400% and 1800%, respectively). The increases were statistically significant at the 100 mg/kg/day and 250 mg/kg/day dosage groups. Similarly, tasimelteon caused statistically significant dose-dependent increases in the activity of following enzymes in both male and female rats; testosterone 16β-hydroxylation (CYP2B1/2, up to 2500% and 1100%, respectively), 4-nitrophenol hydroxylation (CYP2E1, up to 160% and 87%, respectively), testosterone 6β-hydroxylation (CYP3A1/2, up to 260% and 640%, respectively), lauric acid 12-hydroxylation (CYP4A1-3, up to 49% at 30%, respectively), 4-methylumbelliferone glucuronidation (up to 130% and 130%, respectively), thyroxine glucuronidation (up to 110% and 84%, respectively) and triiodothyronine glucuronidation (up to 100% and 45%, respectively) when compared to the vehicle control.

The sponsor also conducted several *in vitro* evaluations of tasimelteon and its metabolites as an inducer and inhibitor of human cytochrome P450 enzymes. These studies are summarized below. The sponsor indicated that hepatic enzyme induction of CYPs 1A2 and 2B6 is not expected in humans at the RHD. Tasimelteon (VEC-162; up to concentrations of 326 μM) and its metabolites (M9, M11, M12, M13, or M14; up to 5 μM each) did not impact P-gp function in Caco-2 cells, indicating that the compounds are not P-gp inhibitors at the tested concentrations.

Protocol (b) (4) 063014: *In vitro* Evaluation of VEC-162 as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes

Conducted by (b) (4) dated 3/28/07 [non-GLP]
Drug: VEC-162, (b) (4) lot number 3049118, (b) (4) batch number 800153250, 99.3% purity; 0.1% dimethyl sulfoxide (vehicle)
Three preparations of cultured hepatocytes were treated once daily for three consecutive days with 0, 1, 10 or 100 μM VEC-162. VEC-162 had little or no-effect on CYP1A2 activity. VEC-162 caused concentration-dependent increases in CYP2C8 activity (up to 4.43-fold, [ss]) and CYP3A4/5 activity (up to 2.27- fold, [ss]), which were accompanied by increases in CYP2C8 and CYP3A4 protein levels. VEC-162 was up to 60.3% and 83.2% as effective as rifampin at inducing CYP2C8 and CYP3A4/5 activity,

respectively. VEC-162 caused concentration-dependent reductions in CYP2C9 activity (as much as 70.8%, [ss]), and CYP2C19 activity (as much as 64.9%, [ss]), compared to vehicle. Decreased CYP2C9 activity was not associated with a corresponding decrease in the level of CYP2C9 protein. (The lack of a specific antibody precluded an assessment of CYP2C19 protein levels.)

Protocol (b) (4) 065016: VEC-162: *In Vitro* Evaluation of VEC-162 as an Inhibitor of Human Cytochrome P450 Enzymes

Conducted by (b) (4) dated 8/23/06 [non-GLP]
Drug: VEC-162, (b) (4) lot 800153250, 99.3% purity (HPLC)
VEC-162 caused direct inhibition of CYP2C19 (IC_{50} = 80 μM) and, to a lesser extent, CYP1A2, CYP2C8, CYP2C9, CYP2D6, and CYP3A4/5 (using testosterone 6β-hydroxylation and midazolam 1'-hydroxylation]). There was little to no evidence of time-dependent inhibition by VEC-162 of any of the CYP enzymes evaluated. See the sponsor's summary table, below.

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(b) (4) 065016 VEC-162

Table 3: Summary of Results: *In Vitro* Evaluation of VEC-162 as an Inhibitor of Human CYP Enzymes

		Direct inhibition		Metabolism-dependent inhibition		
		Zero-minute pre-incubation		30-minute pre-incubation		
Enzyme	CYP Reaction	IC_{50} (μM)	Maximum inhibition at 100 μM (%)[a]	IC_{50} (μM)	Maximum inhibition at 100 μM (%)[a]	Evidence of time-dependent inhibition[b]
CYP1A2	Phenacetin *O*-deethylation	> 100 μM	30	> 100 μM	29	little or no
CYP2C8	Amodiaquine *N*-dealkylation	> 100 μM	28	> 100 μM	13	little or no
CYP2C9	Diclofenac 4′-hydroxylation	> 100 μM	12	> 100 μM	26	little or no
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	80 ± 24	55	68 ± 25	61	little or no
CYP2D6	Dextromethorphan *O*-demethylation	> 100 μM	17	> 100 μM	1.2	little or no
CYP3A4/5	Testosterone 6β-hydroxylation	> 100 μM	34	> 100 μM	35	little or no
CYP3A4/5	Testosterone 6β-hydroxylation repeat[c]	> 100 μM	32	> 100 μM	38	little or no
CYP3A4/5	Midazolam 1′-hydroxylation	> 100 μM	28	> 100 μM	34	little or no

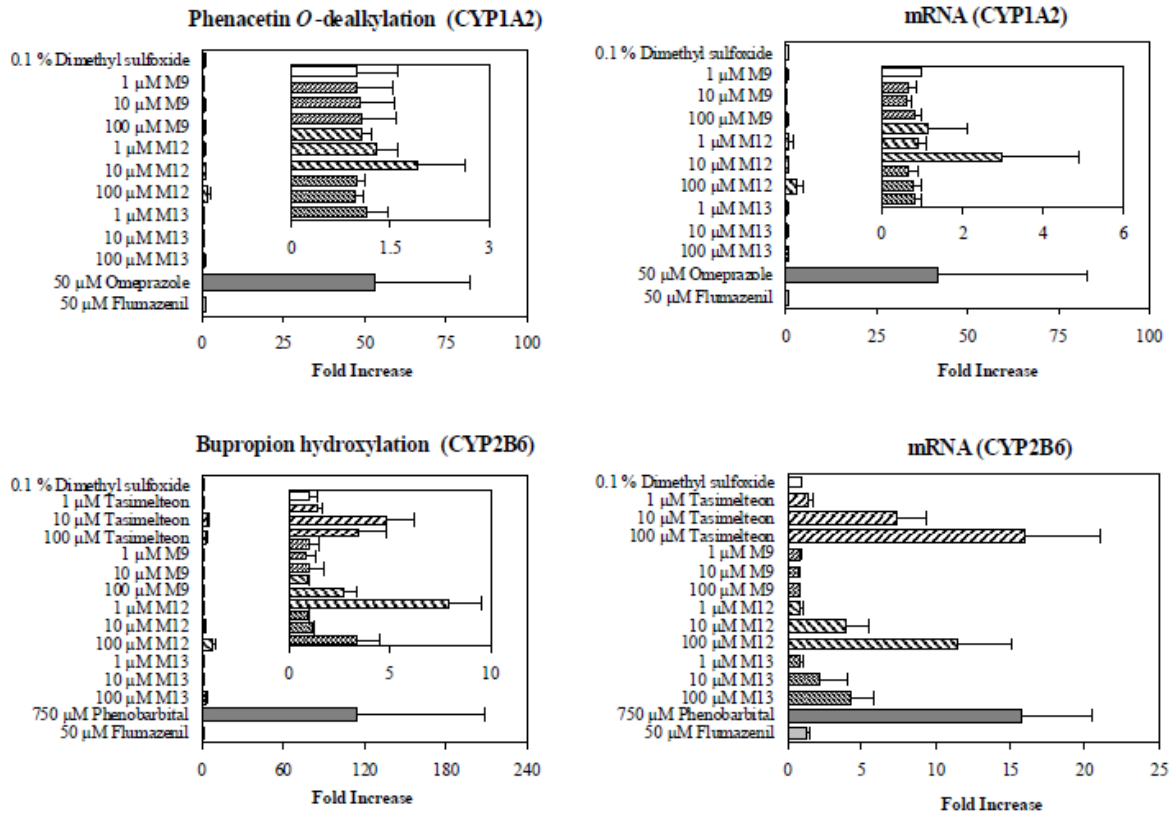
Notes Values were calculated using the average data obtained from duplicates for each incubation condition. The IC_{50} values were calculated using XLfit. For those CYP enzymes where an actual IC50 value was obtained the IC50 value is followed by the standard deviation.
[a] Maximum inhibition (%) is calculated using the following formula and data for the highest concentration of test article for which usable data were collected (results are rounded to two significant figures): Maximum inhibition (%) = 100% – Percent solvent control (see Appendix 4).
[b] Time-dependent inhibition was determined by comparison of IC_{50} values with and without pre-incubation and by visual inspection of the IC_{50} plot.
[c] Experiment was repeated because the metabolism-dependent positive control did not show inhibition after a pre-incubation.
NA Not applicable

Study (b) (4) 123078: *In Vitro* Evaluation of Tasimelteon and Metabolites M9, M12, and M13 as Inducers of Cytochrome P450 Expression in Cultured Human Hepatocytes

Conducted by (b) (4) dated 2/20/13 [non-GLP]
The effects of tasimelteon and/or its most abundant metabolites (M9, M12, and M13) on the expression of cytochrome P450 (CYP) 1A2 and 2B6 enzymes were evaluated. Tasimelteon's potential for inducing CYP1A2 was previously evaluated (Study (b) (4) 063014), and was not included in this study. At concentrations similar to the sponsor-reported "average human maximum plasma concentrations" of 1 to 2.5 μM (C_{max} at 20 mg), neither tasimelteon nor its metabolites appeared to induce CYP1A2 or CYP2B6; however, at higher concentrations (10x to 250x higher) tasimelteon and metabolites M12 and M13 induced CYP2B6 activity (6x, 10x, and 5x for 100 μM tasimelteon, M12, and 13, respectively) and mRNA levels (21x, 16x, and 6x for 100 μM tasimelteon, M12, and 13, respectively). There was a slight suggestion of M12 activity

(3-5x changes at 100 μM) on CYP1A2 activity and mRNA. See the sponsor's summary Figure 9, below.

Figure 9: Mean fold increase: The effects of treating cultured human hepatocytes with tasimelteon, M9, M12, M13 or prototypical inducers on cytochrome P450 (CYP) enzyme activity and mRNA levels



<u>Study [(b) (4)] 12A024: *In Vitro* Evaluation of M9, M12, and M13 as Potential Inhibitors of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes</u>

Conducted by [(b) (4)] dated 2/19/13 [non-GLP]

The ability of three tasimelteon metabolites M9, M12, and M13 to inhibit, *in vitro*, the major CYP enzymes in human liver microsomes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5) was evaluated. M12 showed some effects on CYPs 2C9 and 2C19 (see summary table from the sponsor's report, below).

Table 7: Summary of results: *In vitro* evaluation of M9, M12 and M13 as inhibitors of human CYP enzymes

Enzyme	Enzyme reaction	M9			M12			M13		
		[a]IC_{50} (µM) Zero-minute pre-incubation	[a]IC_{50} (µM) 30-min pre-incubation	[b]Potential for TDI	[a]IC_{50} (µM) Zero-minute pre-incubation	[a]IC_{50} (µM) 30-min pre-incubation	[b]Potential for TDI	[a]IC_{50} (µM) Zero-minute pre-incubation	[a]IC_{50} (µM) 30-min pre-incubation	[b]Potential for TDI
CYP1A2	Phenacetin *O*-dealkylation	>100	>100	No	>100	>100	Yes	>100	>100	No
CYP2B6	Efavirenz 8-hydroxylation	>100	>100	No	>100	>100	No	>100	>100	No
CYP2C8	Amodiaquine *N*-dealkylation	>100	>100	No	>100	>100	No	>100	>100	No
CYP2C9	Diclofenac 4′-hydroxylation	>100	>100	No	>100	[c]85, [d]>100	[c]Yes/ [d]No	>100	>100	No
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	>100	>100	No	>100	[c]24, [d]92	[c]Yes/ [d]Yes	>100	>100	No
CYP2D6	Dextromethorphan O-demethylation	>100	>100	No	>100	>100	No	>100	>100	No
CYP3A4/5	Midazolam 1′-hydroxylation	>100	>100	No	84	61	No	>100	>100	No

[a] Mean data (i.e., percent of control activity) obtained from triplicate samples for each test article concentration were used to calculate IC_{50} values.
[b] Time-dependent inhibition (TDI) was determined by comparison of IC_{50} values obtained with and without preincubation, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC_{50} plots.
[c] Result as determined using the initial lot of M12 received.
[d] Result as determined using a second, purer batch of M12.

(b) (4) **STUDY NUMBER: 10VNDAP1R1: P-gp Interaction Assessment of the Customer's Test Compound (VEC-162) and Metabolites**

Conducted by (b) (4) dated 9/6/11 [non-GLP]

The potential of VEC-162 and its metabolites (M9, M11, M12, M13, and M14) to be P-gp substrates and inhibitors was assessed in Caco-2 cell monolayers. The efflux ratios of all test compounds were less than 2 at the tested concentrations, indicating that the compounds are not P-gp substrates. The presence of VEC-162 (up to concentrations of 326 µM) and M9, M11, M12, M13, or M14 (up to 5 µM each) did not impact P-gp function in Caco-2 cells, indicating that the compounds are not P-gp inhibitors at the tested concentrations. The maximum concentrations tested were based on the tolerability of the Caco-2 monolayers.

In particular, the understanding of the metabolism of tasimelteon has been evolving over the last several years. Knowledge about the number, identity, and relative abundance of *in vivo* VEC-162 metabolites in humans and the relevant toxicologic species has changed considerably since the beginning of the drug's development, and much of the data have been obtained relatively recently. Notably, complete steady state metabolism data are available for rat (tasimelteon and M11 only for mouse), but only single dose metabolism data were provided for monkey. Tasimelteon and M11 exposures were determined at only 200 mg/kg PO in rabbit. Although several metabolites will be discussed, this review will focus on interspecies comparisons of the only the major human circulating metabolites (M9, M12, and M13, identified by FDA's Clinical Biopharmaceutics staff).

In study PHZ00011 (and addendum study (b) (4) 07816), a mass balance study of a single 25 mg/kg [^{14}C]-VEC-162 dose administered PO by oral gavage to male Sprague-Dawley rats demonstrated that the highest mean radioactivity in plasma was observed at 1 hr post-dose, and mean concentrations of radioactivity decreased to below the quantifiable limit within 72 hr. Elimination of radioactivity in urine and feces accounted for 75.31% and 21.64% of the dose, respectively, through 72 hours after dosing. Approximately 91% of the dose was eliminated by 24 hours after dosing. Cage residue samples contained a combined total of 2.62% of the dose, and carcasses contained a mean total of 0.63% of the dose. Tasimelteon and human metabolites M3, M9, M12, M13, and M14 were confirmed in rat plasma. It is noted that M11 was not observed to be present in rat plasma. See the sponsor's summary Table S-2 below.

Table S-2 Concentrations (VEC-162-ng Eq./g) of VEC-162 and Its Metabolites in 0.5, 1, 2, 4, and 8 Hour Rat Plasma and Estimated $AUC_{0\text{-}8hr}$

Entity	Concentration (VEC-162-ng Eq./g)					$AUC_{0\text{-}8\ hr}$	
Metabolites	0.5 hr	1 hr	2 hr	4 hr	8 hr	hr*ngEq/g	%[c]
Total Radioactivity	13180	18940	10700	6590	1070	58755	100.00
VEC-162	3585	4943	1605	432	9.84	9223	15.7
M3	228	455	435	302	110	2234	3.80
M9	1141	1051	416	117	55.0	2444	4.16
M12	4468	7159	5896	3829	254	28443	48.4
M13	179	81.4	49.2	29.7	11.8	337	0.57
M14	232	432	155	85.0	ND	928	1.58

In an IV study (Study 8267772), tasimelteon was administered to male Sprague-Dawley rats in a single dose of 0.25 to 5 mg/kg. For tasimelteon, the observed clearance was high (34-75 mL/min/kg) and the volume of distribution greatly exceeded total blood volume (1240-2070 mL/kg; approximately 6-10x total blood volume, cf. Derelanko, 2000). The observed elimination half-lives were short (means ranging from 0.25- 0.69 hr). Exposure was generally linear (based on AUC), but a potential for enterohepatic recirculation at the 5 mg/kg dose was noted (based on the apparent second increase in plasma concentrations at 12 hr). Metabolites M9, M12, M13, and M14 demonstrated short T_{max} (0.08- 0.5 hr). The observed half-lives for all metabolites were short. A dose-responsive, but not dose–proportional, increase in C_{max} and AUC was reported for all metabolites (see the sponsor's summary figures, below).

Figure 1: **Mean Concentrations of tasimelteon and Metabolites in Plasma Following Intravenous Administration of 0.25 mg/kg tasimelteon to Male Sprague Dawley Rats**

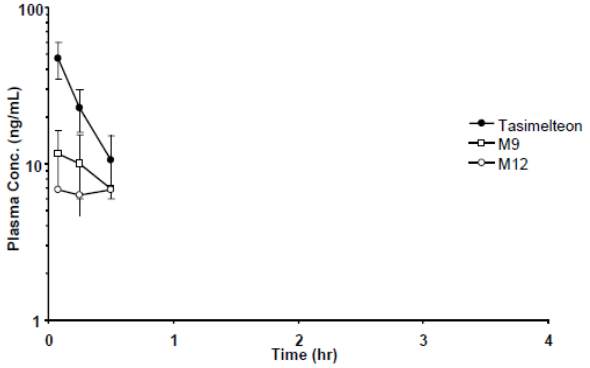


Figure 2: **Mean Concentrations of tasimelteon and Metabolites in Plasma Following Intravenous Administration of 0.50 mg/kg tasimelteon to Male Sprague Dawley Rats**

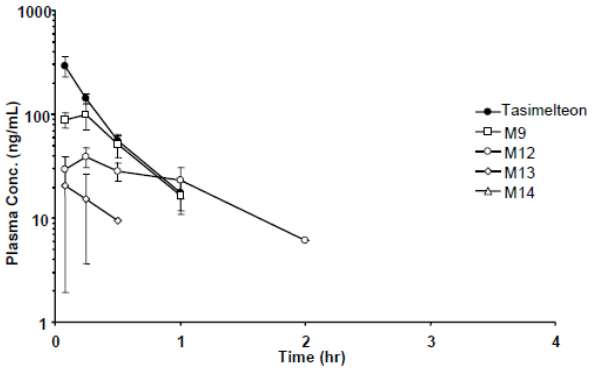


Figure 3: **Mean Concentrations of tasimelteon and Metabolites in Plasma Following Intravenous Administration of 1.0 mg/kg tasimelteon to Male Sprague Dawley Rats**

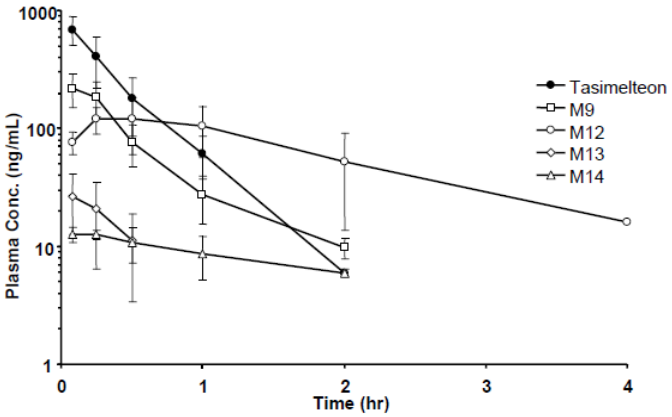


Figure 4: **Mean Concentrations of tasimelteon and Metabolites in Plasma Following Intravenous Administration of 2.5 mg/kg tasimelteon to Male Sprague Dawley Rats**

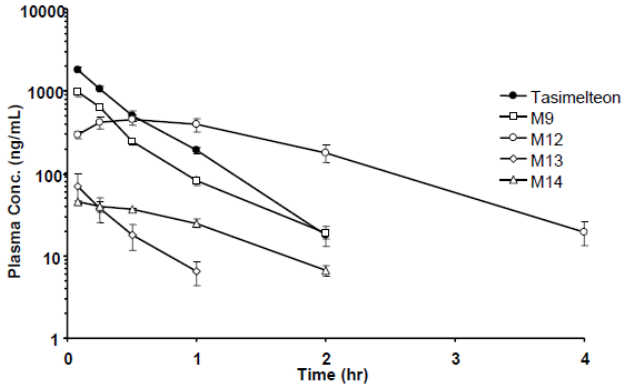
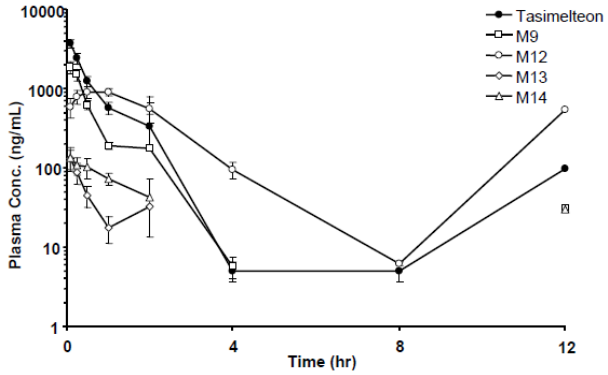


Figure 5: **Mean Concentrations of tasimelteon and Metabolites in Plasma Following Intravenous Administration of 5.0 mg/kg tasimelteon to Male Sprague Dawley Rats**



Steady state plasma exposure to tasimelteon (and metabolites M9, M12, M13, and M14) was investigated in Sprague-Dawley rats (3/sex/gp) following 29 days of daily oral gavage administration of 25, 10, 250, or 500 mg/kg VEC-162 (GLP, with the exception of M3 data, Study TAJ0014). PK data for M3 were unvalidated. Tasimelteon was tolerated at doses up to 250 mg/kg; underactive behavior, reduced body temperature (as judged by coolness to touch), partially closed eyelid(s), unsteady gait, irregular breathing (F), piloerection (F), abnormal gait (F), and uncoordinated unsteady gait (F) were observed at 500 mg/kg. The rate and extent of systemic exposures to tasimelteon and the characterized metabolites were generally non-linear and dose-dependent. Usually, the AUC_{24} values were lower than predicted based on a linear relationship. Accumulation was suggested for tasimelteon (500 mg/kg; 1.5x in F only), M9 (250 and 500 mg/kg; 1.5x and ~3x, respectively) and M13 (250 and 500 mg/kg; 2x and 4.5x, respectively, in F only). (Accumulation for M3 in females ranged from 2x to 29x.) Summary PK data for tasimelteon, M9, M12, and M13 are presented below from the sponsor's submission, as well as metabolite ratios (metabolite AUC/ tasimelteon AUC) for M9, M12, and M13. (For reference, sponsor-provided human AUC plasma exposures to tasimelteon, M9, M12, and M13 at the RHD of 20 mg were 411.4, 379.4, 655.2, and 393.7 ng*hr/mL, respectively.)

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Table 16 Pharmacokinetic parameters derived from plasma tasimelteon concentrations in male rats administered tasimelteon by oral gavage over 29 days at nominal doses of 25, 100, 250 and 500 mg/kg/day

Nominal dose (mg/kg)	Animal number	Day 1						Day 29					
		C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)	C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)
25	1	3350	4	BLQ	18400	0.5825	1.2	3790	2	BLQ	12700	0.6327	1.1
25	2	4200	4	BLQ	29500	1.0382	0.7	2990	2	BLQ	11200	0.5092	1.4
25	3	4870	0.5	BLQ	17100	0.3855	1.8	2080	2	BLQ	8010	0.4352	1.6
Mean		4140	4[a]	-	21700	0.6687	1.0[b]	2950	2[a]	-	10600	0.5257	1.3[b]
Sd		760		-	6810	0.3348		860		-	2400	0.0998	
100	4	17700	4	5.90	121000	0.4834	1.4	3160	0.5	BLQ	13000	0.7895	0.9
100	5	7520	8	BLQ	91000	[c]	[c]	8030	0.5	BLQ	26100	[c]	[c]
100	6	8610	8	BLQ	75800	[c]	[c]	4620	8	BLQ	34700	[c]	[c]
Mean		11300	8[a]	-	95900	-	-	5270	0.5[a]	-	24600	-	-
Sd		5600		-	23000			2500		-	10900		
250	7	12100	6	215	163000	0.2601	2.7	5280	6	11.8	41900	[c]	[c]
250	8	20700	0.5	3220	216000	0.0855	8.1	10600	4	6.7	94000	0.4529	1.5
250	9	15100	6	34.2	168000	0.3867	1.8	12700	8	5.12	72500	[c]	[c]
Mean		16000	6[a]	1160	182000	0.2441	2.8[b]	9530	6[a]	7.87	69500	-	-
Sd		4400		1790	29000	0.1512		3820		3.49	26200		
500	10	21400	0.5	5820	181000	[c]	[c]	8390	2	6.43	73800	0.4387	1.6
500	11	25800	1	6880	219000	[c]	[c]	14400	8	165	195000	0.2957	2.3
500	12	22800	0.5	14460	221000	[c]	[c]	25300	0.5	12.8	129000	0.4231	1.6
Mean		23300	0.5[a]	9050	207000	-	-	16000	2[a]	61.4	133000	0.3858	1.8[b]
Sd		2200		4710	23000			8600		89.7	61000	0.0784	

[a] Median for T_{max}
[b] Calculated as ln2/mean k
[c] Could not be reliably estimated from the data
Means and Sds calculated from rounded values

Table 17 Pharmacokinetic parameters derived from plasma tasimelteon concentrations in female rats administered tasimelteon by oral gavage over 29 days at nominal doses of 25, 100, 250 and 500 mg/kg/day

Nominal dose (mg/kg)	Animal number	Day 1						Day 29					
		C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)	C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)
25	13	4670	2	BLQ	20100	[c]	[c]	1520	0.5	BLQ	3900	1.0442	0.7
25	14	6190	0.5	BLQ	24000	[c]	[c]	7180	1	BLQ	20300	0.6373	1.1
25	15	10100	0.5	BLQ	17200	0.8773	0.8	4260	2	BLQ	13600	0.9874	0.7
Mean		6990	0.5[a]	-	20400	-	-	4320	1[a]	-	12600	0.8896	0.8[b]
Sd		2800		-	3400			2830		-	8200	0.2204	
100	16	17600	0.5	1060	106000	0.0975	7.1	19400	0.5	BLQ	84700	[c]	[c]
100	17	10800	0.5	277	106000	0.2103	3.3	15500	0.5	BLQ	87000	0.1592	4.4
100	18	12600	4	BLQ	104000	[c]	[c]	11400	0.5	BLQ	45200	0.4122	1.7
Mean		13700	0.5[a]	446	105000	0.1539	4.5[b]	15400	0.5[a]	-	72300	0.2857	2.4[b]
Sd		3500		550	1000			4000		-	23500	-	
250	19	19800	0.5	1540	152000	0.0931	7.4	15800	0.5	BLQ	199000	[c]	[c]
250	20	14300	0.5	3980	133000	[c]	[c]	10400	0.5	5.39	127000	[c]	[c]
250	21	37900	0.5	7060	288000	[c]	[c]	10100	0.5	7.59	132000	[c]	[c]
Mean		24000	0.5[a]	4190	191000	-	-	12100	0.5[a]	4.33	153000	-	-
Sd		12300		2770	85000			3200		3.91	40200		
500	22	22500	0.5	4040	136000	[c]	[c]	16000	0.5	4030	171000	[c]	[c]
500	23	17200	0.5	8690	147000	[c]	[c]	22600	0.5	78.8	214000	[c]	[c]
500	24	26100	0.5	5890	195000	[c]	[c]	35700	0.5	616	355000	[c]	[c]
Mean		21900	0.5[a]	6210	159000	-	-	24800	0.5[a]	1570	247000	-	-
Sd		4500		2340	31000			10000		2140	96300		

[a] Median for T_{max}
[b] Calculated as ln2/mean k
[c] Could not be reliably estimated from the data
Means and Sds calculated from rounded values

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Table 18 Pharmacokinetic parameters derived from plasma M9 concentrations in male rats administered tasimelteon by oral gavage over 29 days at nominal doses of 25, 100, 250 and 500 mg/kg/day

Nominal dose (mg/kg)	Animal number	Day 1						Day 29					
		C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)	C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)
25	1	1150	0.5	BLQ	3220	0.3919	1.8	1460	0.5	BLQ	3270	0.4147	1.7
25	2	1440	0.5	BLQ	3520	0.3501	2.0	895	0.5	9.19	3330	0.1440	4.8
25	3	1620	0.5	BLQ	2840	0.3778	1.8	715	0.5	BLQ	2700	0.4298	1.6
Mean		1400	0.5[a]	-	3190	0.3733	1.9[b]	1020	0.5[a]	-	3100	0.3295	2.1[b]
Sd		240		-	340	0.0213		390		-	350	0.1608	
100	4	1580	0.5	39.8	8360	0.1623	4.3	2830	0.5	BLQ	6550	0.3222	2.2
100	5	1230	0.5	5.02	6110	0.2867	2.4	1970	0.5	9.81	6080	0.2156	3.2
100	6	3280	0.5	34.2	12000	0.2031	3.4	2750	0.5	8.76	14100	0.3078	2.3
Mean		2030	0.5[a]	26.3	8820	0.2174	3.2[b]	2520	0.5[a]	5.39	8910	0.2819	2.5[b]
Sd		1100		18.7	2970	0.0634		480		0.74	4500	0.0578	
250	7	1890	0.5	239	12300	[c]	[c]	2420	1	12.5	16100	0.2839	2.4
250	8	4000	0.5	603	18600	[c]	[c]	3620	0.5	30.9	21300	0.2427	2.9
250	9	1670	0.5	81.8	9330	0.1092	6.3	2800	8	19.3	21100	0.2941	2.4
Mean		2520	0.5[a]	308	13400	-	-	2950	1[a]	20.9	19500	0.2736	2.5[b]
Sd		1290		267	4700			610		9.3	2900	0.0272	
500	10	4770	0.5	1180	18100	[c]	[c]	4930	0.5	36.6	37900	0.2786	2.5
500	11	4150	0.5	544	15500	[c]	[c]	11000	1	1250	50100	[c]	[c]
500	12	2810	0.5	756	13300	[c]	[c]	10300	0.5	152	41000	0.1708	4.1
Mean		3910	0.5[a]	827	15600	-	-	8740	0.5[a]	480	43000	0.2247	3.1[b]
Sd		1000		324	2400			3320		670	6300		

[a] Median for T_{max}
[b] Calculated as ln2/mean k
[c] Could not be reliably estimated from the data
Means and Sds calculated from rounded values

Table 19 Pharmacokinetic parameters derived from plasma M9 concentrations in female rats administered tasimelteon by oral gavage over 29 days at nominal doses of 25, 100, 250 and 500 mg/kg/day

Nominal dose (mg/kg)	Animal number	Day 1						Day 29					
		C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)	C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)
25	13	1350	0.5	BLQ	3430	0.4127	1.7	349	0.5	BLQ	1160	[c]	[c]
25	14	2130	0.5	BLQ	4290	0.2963	2.3	2830	1	BLQ	7200	0.2493	2.8
25	15	3570	0.5	10.3	4180	[c]	[c]	1930	0.5	21.5	4290	[c]	[c]
Mean		2350	0.5[a]	-	3970	0.3545	2.0	1700	0.5[b]	-	4220	-	-
Sd		1130		-	470			1260		-	3020		
100	16	1980	0.5	182	9260	[c]	[c]	2490	0.5	8.39	9950	0.2603	2.7
100	17	2550	0.5	68.9	8850	0.1187	5.8	2300	0.5	5.71	10100	0.2981	2.3
100	18	3140	0.5	10.2	10300	0.2425	2.9	2910	0.5	BLQ	8470	0.2006	3.5
Mean		2560	0.5[a]	87.0	9470	0.1806	3.8[b]	2570	0.5[b]	4.70	9510	0.2530	2.7[b]
Sd		580		87.3	750			310		4.29	901	0.0492	
250	19	4210	0.5	329	15300	0.0262	26.5	3780	0.5	44.1	27500	[c]	[c]
250	20	2590	0.5	512	12800	[c]	[c]	9850	0.5	49.5	22600	0.1852	3.7
250	21	7530	0.5	802	23700	[c]	[c]	2650	0.5	53.6	23900	0.2176	3.2
Mean		4780	0.5[a]	548	17300	-	-	5430	0.5[b]	49.1	24700	0.2014	3.4[b]
Sd		2520		239	5700			3870		4.8	2500	0.0229	
500	22	4500	0.5	651	14300	[c]	[c]	3970	0.5	1480	44300	0.0226	30.7
500	23	2240	0.5	517	11400	[c]	[c]	8470	0.5	225	44000	[c]	[c]
500	24	5170	0.5	719	16500	[c]	[c]	4010	0.5	703	51300	[c]	[c]
Mean		3970	0.5[a]	629	14100	-	-	5480	0.5[b]	803	46500	-	-
Sd		1540		103	2600			2590		633	4100	-	-

[a] Median for T_{max}
[b] Calculated as ln2/mean k
[c] Could not be reliably estimated from the data
Means and Sds calculated from rounded values

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Table 20 **Pharmacokinetic parameters derived from plasma M12 concentrations in male rats administered tasimelteon by oral gavage over 29 days at nominal doses of 25, 100, 250 and 500 mg/kg/day**

Nominal dose (mg/kg)	Animal number	Day 1						Day 29					
		C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)	C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)
25	1	3230	4	BLQ	23100	0.6863	1.0	4510	2	BLQ	21000	0.7044	1.0
25	2	4100	6	BLQ	35000	0.2959	2.3	5520	2	BLQ	36900	0.3001	2.3
25	3	3330	2	BLQ	23000	0.5518	1.3	3540	2	BLQ	22000	0.5756	1.2
Mean		3550	4[a]	-	27000	0.5113	1.4[b]	4520	2[a]	-	26600	0.5267	1.3[b]
Sd		480		-	6900	0.1983		990		-	8900	0.2065	
100	4	13000	4	55.2	158000	[c]	[c]	7320	0.5	BLQ	41900	0.4972	1.4
100	5	8620	12	BLQ	128000	[c]	[c]	8610	0.5	BLQ	62600	0.5997	1.2
100	6	8320	12	118	113000	[c]	[c]	7530	8	7.65	85300	0.4472	1.5
Mean		9980	12[a]	57.7	133000	-	-	7820	0.5[a]	-	63300	0.5147	1.3[b]
Sd		2620		59.0	23000			690		-	21710	0.0777	
250	7	13900	12	1750	210000	[c]	[c]	10200	2	BLQ	91400	[c]	[c]
250	8	18800	12	12500	305000	[c]	[c]	17900	4	BLQ	228000	[c]	[c]
250	9	14800	12	624	215000	[c]	[c]	22600	8	BLQ	148000	[c]	[c]
Mean		15800	12[a]	4960	243000	-	-	16900	4[a]	-	156000	-	-
Sd		2600		6560	53000			6300		-	69000		
500	10	14500	12	13300	279000	[c]	[c]	16600	2	BLQ	232000	[c]	[c]
500	11	17800	24	17800	335000	[c]	[c]	19200	12	927	296000	[c]	[c]
500	12	18900	24	18900	284000	[c]	[c]	24000	0.5	77.5	245000	0.3445	2.0
Mean		17100	24[a]	16700	299000	-	-	19900	2[a]	335	258000	-	-
Sd		2300		3000	31000			3800		514	34000		

[a] Median for T_{max}
[b] Calculated as ln2/mean k
[c] Could not be reliably estimated from the data
Means and Sds calculated from rounded values

Table 21 **Pharmacokinetic parameters derived from plasma M12 concentrations in female rats administered tasimelteon by oral gavage over 29 days at nominal doses of 25, 100, 250 and 500 mg/kg/day**

Nominal dose (mg/kg)	Animal number	Day 1						Day 29					
		C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)	C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)
25	13	6630	4	BLQ	52500	0.4420	1.6	5970	4	BLQ	24000	0.7804	0.9
25	14	6680	8	BLQ	87400	[c]	[c]	10200	4	BLQ	77800	0.2883	2.4
25	15	7390	2	BLQ	51000	0.4652	1.5	9220	2	BLQ	52100	0.6606	1.0
Mean		6900	4[a]	-	63600	0.4536	1.5[b]	8460	4[a]	-	51300	0.5764	1.2[b]
Sd		430		-	20600			2210		-	26900	0.2566	
100	16	19700	12	11100	336000	[c]	[c]	16300	6	11.3	223000	0.4753	1.5
100	17	13000	12	10500	230000	[c]	[c]	13300	4	BLQ	186000	[c]	[c]
100	18	13900	8	167	187000	0.2930	2.4	14700	4	BLQ	142000	0.1819	3.8
Mean		15500	12[a]	7260	251000	-	-	14800	4[a]	-	184000	0.3286	2.1[b]
Sd		3600		6150	77000			1500		-	41000		
250	19	26200	12	14700	418000	[c]	[c]	20500	12	8.46	317000	[c]	[c]
250	20	19500	24	19500	375000	[c]	[c]	21100	12	10.8	276000	[c]	[c]
250	21	24200	8	21500	445000	[c]	[c]	16200	12	47.9	246000	[c]	[c]
Mean		23300	12[a]	18600	413000	-	-	19300	12[a]	22.39	280000	-	-
Sd		3400		3500	35000			2700		22.13	36000		
500	22	16700	24	16700	322000	[c]	[c]	21700	12	11200	380000	[c]	[c]
500	23	24100	24	24100	398000	[c]	[c]	25000	12	426	377000	[c]	[c]
500	24	19100	24	19100	372000	[c]	[c]	39300	12	2770	577000	[c]	[c]
Mean		20000	24[a]	20000	364000	-	-	28700	12[a]	4799	445000	-	-
Sd		3800		3800	39000			9350		5666	115000		

[a] Median for T_{max}
[b] Calculated as ln2/mean k
[c] Could not be reliably estimated from the data
Means and Sds calculated from rounded values

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Table 22 **Pharmacokinetic parameters derived from plasma M13 concentrations in male rats administered tasimelteon by oral gavage over 29 days at nominal doses of 25, 100, 250 and 500 mg/kg/day**

Nominal dose (mg/kg)	Animal number	Day 1						Day 29					
		C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)	C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)
25	1	50.4	0.5	BLQ	268	0.4655	1.5	53.9	2	BLQ	202	0.5651	1.2
25	2	278	0.5	BLQ	869	[c]	[c]	184	0.5	BLQ	679	0.3597	1.9
25	3	123	0.5	BLQ	303	0.3430	2.0	47.7	2	BLQ	188	0.3050	2.3
Mean		150	0.5[a]	-	480	0.4043	1.7[b]	95.2	2[a]	-	356	0.4099	1.7[b]
Sd		116		-	337			77.0		-	280	0.1371	
100	4	211	8	BLQ	2480	[c]	[c]	134	0.5	BLQ	694	[c]	[c]
100	5	363	0.5	BLQ	2380	[c]	[c]	302	0.5	BLQ	884	0.4331	1.6
100	6	129	0.5	BLQ	1120	[c]	[c]	131	8	BLQ	917	[c]	[c]
Mean		234	0.5[a]	-	1990	-	-	189	0.5[a]	-	832	-	-
Sd		119		-	760			98		-	120		
250	7	356	12	25.0	5520	[c]	[c]	492	6	BLQ	4010	[c]	[c]
250	8	206	0.5	107	3220	0.0308	22.5	358	4	BLQ	3240	[c]	[c]
250	9	401	0.5	9.46	3670	0.2088	3.3	867	8	BLQ	4820	[c]	[c]
Mean		321	0.5[a]	47.2	4140	0.1198	5.8[b]	572	6[a]	-	4020	-	-
Sd		102		52.4	1220			264		-	790		
500	10	437	24	437	7460	[c]	[c]	1350	2	BLQ	12100	[c]	[c]
500	11	451	0.5	187	3550	[c]	[c]	478	8	7.27	7170	0.2799	2.5
500	12	913	0.5	475	6780	[c]	[c]	720	0.5	BLQ	5060	[c]	[c]
Mean		600	0.5[a]	366	5930	-	-	849	2[a]	-	8110	-	-
Sd		271		156	2090			450		-	3610		

[a] Median for T_{max}
[b] Calculated as ln2/mean k
[c] Could not be reliably estimated from the data
Means and Sds calculated from rounded values

Table 23 **Pharmacokinetic parameters derived from plasma M13 concentrations in female rats administered tasimelteon by oral gavage over 29 days at nominal doses of 25, 100, 250 and 500 mg/kg/day**

Nominal dose (mg/kg)	Animal number	Day 1						Day 29					
		C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)	C_{max} (ng/mL)	T_{max} (h)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (1/h)	$t_{½}$ (h)
25	13	74.9	0.5	BLQ	208	0.3749	1.8	27.7	2	BLQ	58.7	[c]	[c]
25	14	174	0.5	BLQ	378	[c]	[c]	114	1	BLQ	270	0.5860	1.2
25	15	154	0.5	BLQ	203	0.7536	0.9	60.4	0.5	BLQ	147	0.5524	1.3
Mean		134	0.5[a]	-	263	0.5643	1.4	67.4	1[a]	-	159	0.5692	1.2[b]
Sd		52		-	100		0.7	43.6		-	106		
100	16	808	0.5	46.0	2270	[c]	[c]	770	0.5	BLQ	2450	[c]	[c]
100	17	214	0.5	5.77	1250	0.1701	4.1	241	0.5	BLQ	1520	0.1772	3.9
100	18	165	0.5	BLQ	905	[c]	[c]	161	0.5	BLQ	823	0.2931	2.4
Mean		396	0.5[a]	17.3	1480	-	-	391	0.5[a]	-	1600	0.2352	2.9[b]
Sd		358		25.1	710			331		-	820		
250	19	293	0.5	34.9	1520	[c]	[c]	471	2	BLQ	6370	[c]	[c]
250	20	256	0.5	92.5	1900	[c]	[c]	227	12	BLQ	3140	[c]	[c]
250	21	529	0.5	92.6	2650	[c]	[c]	189	8	BLQ	2810	[c]	[c]
Mean		359	0.5[a]	73.3	2020	-	-	296	8[a]	-	4110	-	-
Sd		148		33.3	580			153		-	1970		
500	22	380	0.5	99.7	1920	[c]	[c]	1080	0.5	307	9910	[c]	[c]
500	23	741	0.5	184	3220	[c]	[c]	1040	0.5	24.0	9960	[c]	[c]
500	24	536	0.5	130	2760	[c]	[c]	1280	0.5	56.0	15300	[c]	[c]
Mean		552	0.5[a]	138	2630	-	-	1130	0.5[a]	129	11700	-	-
Sd		181		43	660			130		155	3100		

[a] Median for T_{max}
[b] Calculated as ln2/mean k
[c] Could not be reliably estimated from the data
Means and Sds calculated from rounded values

Table 34 Metabolite ratios of Metabolite M9 after daily oral administration of tasimelteon to rats

Dose level (mg/kg/day)	Males			Females		
	Rat ID number	Metabolite ratio[a]		Rat ID number	Metabolite ratio[a]	
		Day 1	Day 29		Day 1	Day 29
25	1	0.15	0.23	13	0.15	0.26
	2	0.11	0.23	14	0.16	0.31
	3	0.15	0.30	15	0.21	0.28
	Mean[b]	0.13	0.25	Mean[b]	0.17	0.28
100	4	0.06	0.45	16	0.08	0.10
	5	0.06	0.21	17	0.07	0.10
	6	0.14	0.36	18	0.09	0.17
	Mean[b]	0.08	0.32	Mean[b]	0.08	0.12
250	7	0.07	0.34	19	0.09	0.12
	8	0.08	0.20	20	0.09	0.16
	9	0.05	0.26	21	0.07	0.16
	Mean[b]	0.06	0.26	Mean[b]	0.08	0.15
500	10	0.09	0.45	22	0.09	0.23
	11	0.06	0.23	23	0.07	0.18
	12	0.05	0.28	24	0.07	0.13
	Mean[b]	0.07	0.31	Mean[b]	0.08	0.17

[a] Calculated from AUC_{24} (M9)/AUC_{24} (tasimelteon) and corrected for relative molecular weight
[b] Geometric mean

Table 35 Metabolite ratios of Metabolite M12 after daily oral administration of tasimelteon to rats

Dose level (mg/kg/day)	Males			Females		
	Rat ID number	Metabolite ratio[a]		Rat ID number	Metabolite ratio[a]	
		Day 1	Day 29		Day 1	Day 29
25	1	1.18	1.55	13	2.45	5.78
	2	1.11	2.46	14	3.42	3.60
	3	1.26	2.58	15	2.78	3.60
	Mean[b]	1.18	2.14	Mean[b]	2.86	4.21
100	4	1.23	3.03	16	2.98	2.47
	5	1.32	2.25	17	2.04	2.01
	6	1.40	2.31	18	1.69	2.95
	Mean[b]	1.31	2.51	Mean[b]	2.17	2.45
250	7	1.21	2.05	19	2.58	1.50
	8	1.33	2.28	20	2.65	2.04
	9	1.20	1.92	21	1.45	1.75
	Mean[b]	1.24	2.08	Mean[b]	2.15	1.75
500	10	1.45	2.95	22	2.23	2.09
	11	1.44	1.43	23	2.54	1.65
	12	1.21	1.78	24	1.79	1.53
	Mean[b]	1.36	1.96	Mean[b]	2.17	1.74

[a] Calculated from AUC_{24} (M12)/AUC_{24} (tasimelteon) and corrected for relative molecular weight
[b] Geometric mean

Table 36 Metabolite ratios of Metabolite M13 after daily oral administration of tasimelteon to rats

Dose level (mg/kg/day)	Males			Females		
	Rat ID number	Metabolite ratio[a]		Rat ID number	Metabolite ratio[a]	
		Day 1	Day 29		Day 1	Day 29
25	1	0.01	0.01	13	0.01	0.01
	2	0.03	0.06	14	0.01	0.01
	3	0.02	0.02	15	0.01	0.01
	Mean[b]	0.02	0.03	Mean[b]	0.01	0.01
100	4	0.02	0.05	16	0.02	0.03
	5	0.02	0.03	17	0.01	0.02
	6	0.01	0.02	18	0.01	0.02
	Mean[b]	0.02	0.03	Mean[b]	0.01	0.02
250	7	0.03	0.09	19	0.01	0.03
	8	0.01	0.03	20	0.01	0.02
	9	0.02	0.06	21	0.01	0.02
	Mean[b]	0.02	0.06	Mean[b]	0.01	0.02
500	10	0.04	0.15	22	0.01	0.05
	11	0.02	0.03	23	0.02	0.04
	12	0.03	0.04	24	0.01	0.04
	Mean[b]	0.03	0.06	Mean[b]	0.02	0.05

[a] Calculated from AUC_{24} (M13)/AUC_{24} (tasimelteon) and corrected for relative molecular weight
[b] Geometric mean

Although human metabolite M11 was not detected in rat, it was observed in mouse, pregnant rabbit, and monkey at low levels. Following a single oral gavage dose of 300 mg/kg in mice (non-GLP study (b) (4) 09628), the peak area and % tasimelteon (VEC-162) peak areas of M11, M12, M13, and M14 for mouse are summarized in sponsor's Table 2, below. The sponsor further investigated the steady-state exposures of tasimelteon and metabolite M11 in CD-1 mice (27/sex/gp) after daily oral gavage administration of 100, 300, and 600 mg/kg tasimelteon for 29 days (GLP study TAJ0018). Animals treated with 600 mg/kg tasimelteon showed mortality and adverse clinical signs, and were sacrificed early; adverse clinical signs were also observed at 300 mg/kg. Generally, non-linear, dose-dependent kinetics were observed. The sponsor noted that enterohepatic recirculation was suggested. M11 represented <1% of the total drug-related exposure in mice.

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Table 2

Peak Area and %VEC-162 of M11, M12, M13, and M14 in 0.5-, 1- and 4-Hour Mouse Plasma

Sample Name	Name	Peak Area (counts)	%VEC-162
G1M0.5hP/Ext (X2915:12A), 10 µL	M11	2934223	1.65
	M12	169050854	94.84
	M13	6307246	3.54
	M14	41890673	23.5
	VEC162	178240833	100
G1M1hP/Ext (X2915:7G), 10 µL	M11	778027	0.52
	M12	173883752	116.81
	M13	5722864	3.84
	M14	45229997	30.38
	VEC162	148864326	100
G1M4hP/Ext (X2915:7H), 10 µL	M11	668525	0.55
	M12	184110941	151.99
	M13	4161330	3.44
	M14	35885207	29.62
	VEC162	121135127	100

*See Figure 2.

Only a single dose mass balance study in Cynomolgus monkeys (study PHZ00012, and addendum study (b) (4) 07815) was provided. Following a single 25 mg/kg [^{14}C]-tasimelteon PO dose administered to male and female monkeys, the highest mean radioactivity in plasma was observed at 2 hr post-dose, and the mean concentration of radioactivity decreased to below the quantifiable limit within 24 hr. Urine and feces accounted for approximately 80% and 5% of elimination, respectively; approximately 90% of the dose was eliminated by 24 hours after dosing. All tasimelteon human metabolites (i.e., M1, M3, M8, M9, M11, M12, M13, and M14) were confirmed in monkey plasma. Unchanged [^{14}C]-tasimelteon accounted for 8.91% and 17.7% of the total radioactivity in 0-12 hr male and female monkey plasma, respectively. Metabolite M9 (a phenol-carboxylic acid derivative of tasimelteon) was also the most prominent circulating metabolite in monkeys and accounted for 10.3% (male) and 6.72% (females) of the total radioactivity in 0-12 hr plasma. Metabolite M12 was a predominant metabolite in the female monkey, representing 10.0% of the total radioactivity, while it represented only 3.72% in males. Exposures to the remaining human metabolites were each <50% of the tasimelteon exposure, and less than 5% of the total AUC. See the sponsor's summary Tables S-2, 2, S-3 and 3.

Table S-2 Concentrations (VEC-162-ng Eq./g) of VEC-162 and Its Metabolites in 0.5, 1, 2, 8, and 12 Hour Male Monkey Plasma and Estimated $AUC_{0\text{-}12hr}$

Entity	Concentration (VEC-162-ng Eq./g)					$AUC_{0\text{-}12\ hr}$	
Metabolites	0.5 hr	1 hr	2 hr	8 hr	12 hr	hr*ngEq/g	%
Total Radioactivity	10910	15040	22550	2060	660	107281	100
VEC-162	331	781	2417	69.4	ND	9558	8.91
M3	263	504	907	139	50.0	4480	4.18
M8	410	594	821	100	120	4265	3.98
M9	1597	2035	2381	38.9	70.0	10993	10.3
M12	142	352	925	83.4	ND	3991	3.72
M13	384	766	1012	22.2	ND	4420	4.12
M14	116	247	654	52.7	ND	2796	2.61

Table 2

Concentrations (VEC-162-ng Eq./g) of M1 and M11 in 0.5-, 1-, 2-, 8-, and 12-Hour Male Monkey Plasma and Estimated $AUC_{0\text{-}t\ hr}$

Metabolites	Concentration (VEC-162-ng Eq./g) [b]					$AUC_{0\text{-}t\ hr}$	
	0.5 hr	1 hr	2 hr	8 hr	12 hr	hr*ng Eq/g	%[c]
TRA [a]	10910	15040	22550	2060	660	107281	100
M1	147	217	370	ND	ND	1532	1.43
M11	89.5	212	451	22.2	ND	1893	1.76

[a] Total radioactivity in plasma samples

[b] Concentrations = % of VEC-162 or metabolite in a radio-profile (Table 1) × TRA.

[c] % of the AUC of the total radioactivity in plasma samples.

ND: Non-detectable.

Table S-3 Concentrations (VEC-162-ng Eq./g) of VEC-162 and Its Metabolites in 0.5, 1, 2, 8, and 12 Hour Female Monkey Plasma and Estimated $AUC_{0\text{-}12hr}$

Entity	Concentration (VEC-162-ng Eq./g)					$AUC_{0\text{-}12\ hr}$	
Metabolites	0.5 hr	1 hr	2 hr	8 hr	12 hr	hr*ngEq/g	%
Total Radioactivity	14810	18920	19530	6500	2790	128031	100
VEC-162	2303	3616	4187	840	382	22719	17.7
M3	397	552	678	343	170	5040	3.94
M8	367	730	859	247	153	5279	4.12
M9	2889	2342	1291	150	68.1	8606	6.72
M12	683	1169	1834	1035	379	12813	10.0
M13	1009	819	519	45.5	27.3	3163	2.47
M14	332	552	830	419	160	5580	4.36

Table 3

Concentrations (VEC-162-ng Eq./g) of M1 and M11 in 0.5-, 1-, 2-, 8-, and 12-Hour Female Monkey Plasma and Estimated $AUC_{0\text{-}12\ hr}$

Metabolites	Concentration (VEC-162-ng Eq./g) [b]					$AUC_{0\text{-}12\ hr}$	
	0.5 hr	1 hr	2 hr	8 hr	12 hr	hr*ngEq/g	%[c]
TRA [a]	14810	18920	19530	6500	2790	128031	100
M1	158	282	234	86	41	1540	1.20
M11	194	392	314	48	ND	1731	1.35

[a] Total radioactivity in plasma samples.

[b] Concentrations = % of VEC-162 or metabolite in a radio-profile (Table 1) × TRA.

[c] % of the AUC of the total radioactivity in plasma samples.

ND: Non-detectable.

In pregnant New Zealand rabbits, the plasma exposures of tasimelteon and metabolite M11 only were assessed following administration of 200 mg/kg tasimelteon for 14 days (GD6- GD19; GLP study TAJ0016). See the sponsor's summary PK data, Tables 1 and 2 below. Little to no accumulation was reported for tasimelteon and M11; see sponsor's Table 5, below. Metabolite M11 was observed to be approximately 1% of tasimelteon exposure on GD6, and approximately 3% of tasimelteon exposure on GD19.

Table 1: C_{max} and AUC_8 of Tasimelteon on Day 6 and Day 19 of gestation

Day	C_{max} (ng/mL)	AUC_8 (ng.h/mL)
Day 6	19400 (5200)	113000 (28000)
Day 19	9400 (3000)	46800 (9300)

Table 2: C_{max} and AUC_8 of M11 on Day 6 and Day 19 of gestation

Day	C_{max} (ng/mL)	AUC_8 (ng.h/mL)
Day 6	219 (56)	1330 (340)
Day 19	293 (116)	1470 (460)

Table 5 Accumulation ratios of tasimelteon and M11 after repeated (Day 6 to Day 19 of gestation) daily oral gavage administration of tasimelteon to pregnant female rabbits

Dose level (mg/kg/day)	Rabbit ID number	Accumulation ratio[a] Tasimelteon	M11
200	1	0.40	1.2
	2	0.37	0.95
	3	0.51	1.3
	4	0.40	0.97
	Mean[b]	0.42	1.1

[a] Calculated from AUC_8 (Day 19)/AUC_8 (Day 6)
[b] Geometric mean

6 General Toxicology

6.1 Single-Dose Toxicity

Single doses of BMS-214778 were tested in rats, mice, and monkeys. Generally, single doses of up to 400 mg/kg in rats and mice and 200 mg/kg in monkeys were tolerated. Following a single dose of 1750 mg/kg, rats showed CNS-related signs (i.e., ataxia, ptosis, and hypoactivity on day 1, and loss of righting reflex and prostration on days 2 and 3), labored respiration, and reduction s in body weight. According to the 1/16/98 review by Dr. Atrakchi, the dose of 1750 mg/kg in rats was considered the maximum non-lethal dose; higher doses could not be tested because of the toxicity of PEG-400 and the drug's solubility limit. In mice, a single dose of 1750 mg/kg resulted in mortality, tonic convulsions, "inappropriate locomotion", and "disequilibrium," in addition to the signs previously noted in rats at that dose.

6.2 Repeat-Dose Toxicity

Subchronic studies were conducted in mice, rats and monkeys by the oral route. The vehicle used in all toxicity studies was PEG-400. Most of the subchronic studies were reviewed by Dr. Atrakchi (see review dated 1/16/98). Pertinent results of some of the studies are summarized below. Target organs of toxicity have consistently included the CNS and liver, and kidney in rats. Reproductive organs have also been demonstrated to be a target.

In a 3-month study in mice, BMS-214778 caused mortality at 800 mg/kg, and increased liver weights, dose-related minimal to moderate centrilobular hepatocellular hypertrophy, prostration, labored breathing, and slightly increased serum albumin and total protein (M) at 400 to 600 mg/kg. Relative kidney and prostate weights were decreased in males at 600 mg/kg. The reported no-effect dose was 100 mg/kg.

In a 2-week study, rats showed increased liver weight and size, with dose-dependent centrilobular to panlobular hepatocellular hypertrophy observed at ≥ 100 mg/kg in males and ≥ 200 mg/kg in females. Increased hepatocellular mitoses were observed at 400 mg/kg. Hyaline droplet nephropathy was observed in male rats. Clinical pathology changes were reported at ≥ 200 mg/kg, including increased ALT, globulin, total protein, cholesterol, glucose (F), and triglycerides (F). The reported no-effect dose for 2 weeks was 50 mg/kg. In a 1-month study in rats, increased liver weight and size, minimal to marked centrilobular to panlobular hepatocellular hypertrophy, increased kidney weight (only 400 mg/kg in M), dose-dependent hyaline droplet nephropathy (≥ 100 mg/kg in M), increased heart and adrenal weight (only 400 mg/kg in F), and several changes in clinical chemistry parameters (e.g., increased cholesterol, triglycerides (F), glucose (F), total protein, albumin, globulin, and ALT) were observed at ≥100 mg/kg; most changes were at least partially reversible. Decreased serum urea nitrogen and creatinine were observed. Increased neutrophils (400 mg/kg) and RBC polychromasia and anisocytosis (400 mg/kg in F) were also observed. According to Dr. Atrakchi's review, the NOEL was given as 25 mg/kg.

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In a 1-week study in monkeys, 175 mg/kg resulted in post-dosing emesis and/or salivation, decreased body weight and food consumption, and mild changes in clinical pathology parameters (including increased serum triglycerides (M), glucose (F), ALT (≥100 mg/kg), and fibrinogen, and decreased sodium (M), chloride, and phosphorus). According to Dr. Atrakchi, the NOEL was 50 mg/kg. In a 1-month study, 125 mg/kg was associated with increased liver weights (≥ 45 mg/kg), decreased spleen weights (M), and decreased SV/prostate and testes weights. According to Dr. Atrakchi's review, the reported no-effect dose was 15 mg/kg, and 45 mg/kg was a LOEL.

Chronic toxicity studies were conducted in rats (6-month) and monkeys (12-month, with an interim assessment at 6 months). These studies were terminated after the in-life portion and summary and/or interim reports were issued; upon restarting development, the sponsor contracted CROs to complete the histopathology assessments for these studies. (The rat fertility study was also completed in this manner.) However, it appears that GLP and QA conditions for these studies has been assured, except for the lack of a separate, signed pathology report for the 6-month rat toxicity study (this has been requested).

The chronic toxicity studies demonstrated CNS, hepatic, renal, endocrine, and reproductive effects. These effects generally reflected toxicities previously seen in acute and subchronic toxicity studies, including: CNS signs (e.g., convulsions, hypoactivity, ataxia, labored breathing), body weight reduction, altered hematology parameters (e.g., increased neutrophils, reticulocytes, fibrinogen, RBC polychromasia), altered clinical chemistry parameters (increased ALT, globulin, total proteins, total cholesterol, glucose, and triglycerides), liver enlargement and histopathology (e.g., hepatocellular centrilobular to panlobular hypertrophy, increased hepatocellular mitoses), increased kidney weight and histopathology (hyaline droplet nephropathy), decreased spleen weight, decreased male reproductive organ weights (seminal vesicles, prostate, testes), and increased uterine weight. Also of note, from Dr. Atrakchi's review: a previous finding of eyelid edema and mild dyspnea after tasimelteon (in 50% PEG) dosing in monkeys was dismissed after it was demonstrated to not be reproducible.

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Study Title: BMS-214778: SIX-MONTH ORAL TOXICITY STUDY IN RATS

Study no.: (b) (4) # TAJ0007 /064164; BMS 98348

Study report location: EDR

Conducting laboratory and location: ***In life***
BMS Pharmaceutical Research Institute,
Dept. of Toxicology and Pathology,
Mt Vemon, Indiana

TK
BMS Dept of Metabolism and PK
Princeton, New Jersey

Histopathology (slide reading) & Report
(b) (4)

Date of study initiation: 10/27/98

GLP compliance: Yes (b) (4) (BMS- FDA)

QA statement: Yes (b) (4) (BMS- FDA)

Drug, lot #, and % purity: BMS-214778, batch N031C-214778-01, 99.3%

The in-life portion of this study was completed by BMS. A decision was made by the previous sponsor (BMS) to terminate the project as of 10/27/99; however, the current sponsor asked (b) (4) (12/18/06) to complete the study (histopathology evaluation and study report). Audited formulation analyses and TK reports were supplied by BMS on 3/9/07. The study was completed 7/3/07. Note: there is no separate, signed pathology report. The TK report (signed in 1999; Annex 5) was amended in 2007 ("Amendment 1") to specify that it was audited for US GLP compliance. The NOAEL is 5 mg/kg/day, based on findings in the liver, kidney, and spleen.

Methods (see summary tables from the sponsor, below)

Frequency of dosing: QD

Route of administration: PO, by gavage

Formulation/Vehicle: PEG-400

Species/Strain: Hsd:Sprague Dawley® SD® rats
(b) (4)

Age: ~7 weeks

Weight: M: 198-275 g; F: 157-209 g

Dosed fasted or fed: Fed (ad libitum)

Unique study design: After Week 3, animals were housed in wire-bottomed cages due to pica in all treated groups.

Deviation from study protocol: None reported

Group	Treatment	Dose# (mg/kg/day)	No. of animals Male	Female	Animal numbers Male	Female
1	Control	0	20	20	1101-1120	2101-2120
2	BMS-214778	5	20	20	1201-1220	2201-2220
3	BMS-214778	50	20	20	1301-1320	2301-2320
4	BMS-214778	500	20	20*	1401-1420	2401-2421

Expressed in terms of the free drug.
* 4F 2416 was killed on Day 3 and replaced with 4F 2421.

Group	Treatment	Dose (mg/kg/day)	Concentration (mg/mL) of active	Concentration (mg/mL) as supplied*	Volume dose (mL/kg)
1	Control	0	0	0	3
2	BMS-214778	5	1.7	1.7	3
3	BMS-214778	50	16.7	16.9	3
4	BMS-214778	500	166.7	168.4	3

* - a conversion factor of x1.01 was used to account for the purity of the test substance.

Observations and Results

Dosing Solution Analysis [Weeks 1, 6, &12]

All formulations were homogenous and stable (at ambient temperature for two days and stored at approximately 4°C for 15 days). The mean concentrations of BMS-214778 in the formulations during Weeks 1, 6 and 12 were ±5% of nominal.

Mortality [Twice daily]

There was only one drug-related death (HDF) on D3.

HDF (#2416) was sacrificed moribund on D3. Clinical signs included tonic convulsions, lacrimation, prolonged extension of limbs, hypoactivity, labored respiration, recumbency, ataxia, loss of righting reflex, hunched posture, and coolness to touch. No clinical pathology findings were reported (although the data were not provided). The sponsor reported no drug-related macroscopic or microscopic findings; however, the following findings were reported in this animal: glandular stomach (minimal, focal mucosal erosion), liver (slight centrilobular hepatocyte hypertrophy, with a prominent increase in mitotic activity), kidney (minimal cortical tubular basophilia), spleen (slight decreased cellularity), and reproductive organ (epithelial mucinification) changes. The animal was replaced with HDF #2421.

Clinical Signs [Daily; detailed examination weekly]

No signs were reported at LD or MD.

At HD, clinical signs reflecting effects on the central nervous system occurred throughout the treatment period but were most prevalent during the first two weeks. A single HDF was sacrificed moribund because of the severity of the clinical signs (see

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Mortality). HDF were more often affected than HDM, particularly during the first four days of treatment; signs included (number affected; F/M): hypoactivity (19/12), labored respiration (16/6), ataxia (17/8), loss of righting reflex (11/0), extension of the limbs (14/1), recumbency (16/4), tremors (4/1), and convulsions (5/0).

In HDF, tonic convulsions were observed on D3 (#2416; early mortality), D6 (#2407), D36 (#2408 and #2413), and on D3, 76, 113, and 120 (#2415). In animal #2408, clonic convulsions were observed on D4 and D5.

There was evidence of dose-related pica in the treated animals; increased incidence of bedding material in the feces was observed on D12 (0, 3, 4, 10 in control, LD, MD, and HD males; 1, 0, 6, 15 in control, LD, MD, and HD females). The caging was changed to wire-bottomed cages from D12 on to prevent ingestion of bedding.

A few other signs were noted at lower incidence. Salivation was observed in 3 HDM and 2 HDF. Red discoloration of the muzzle was observed in 4 HDM and 1 HDF. Chromodacryorrhea was observed in 1 conM, 1 MDF, 2 HDM, and 2 HDF. Lacrimation was observed in 2 HDF. Although of questionable relationship to drug, alopecia was observed in 1 female of each treated group.

Body Weights [Weekly]
Throughout the treatment period, average body weights were reduced 6-8% [ss] in treated males, compared to controls. A slight reduction in body weight gain was observed at all doses but was not consistently dose-related (Weeks 1-13: 10%, 11% and 13%; Weeks 1-26: 18, 12, and 13% for LD, MD, and HD males, respectively). In females, slight effects on average body weight gains appeared biphasic. The average body weight gains from Week 1-13 were -11%, -16% and +6%, and were -11%, -6%, and +10% in Weeks 1-26 ([nss] at LD, MD and HD, respectively). There was no significant effect on body weight at Week 26 in females. See the sponsor's Figure 1.

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FIGURE 1

Bodyweight - group mean values versus period of treatment: males

Group	:	1	2	3	4
Compound	:	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	:	0	5	50	500

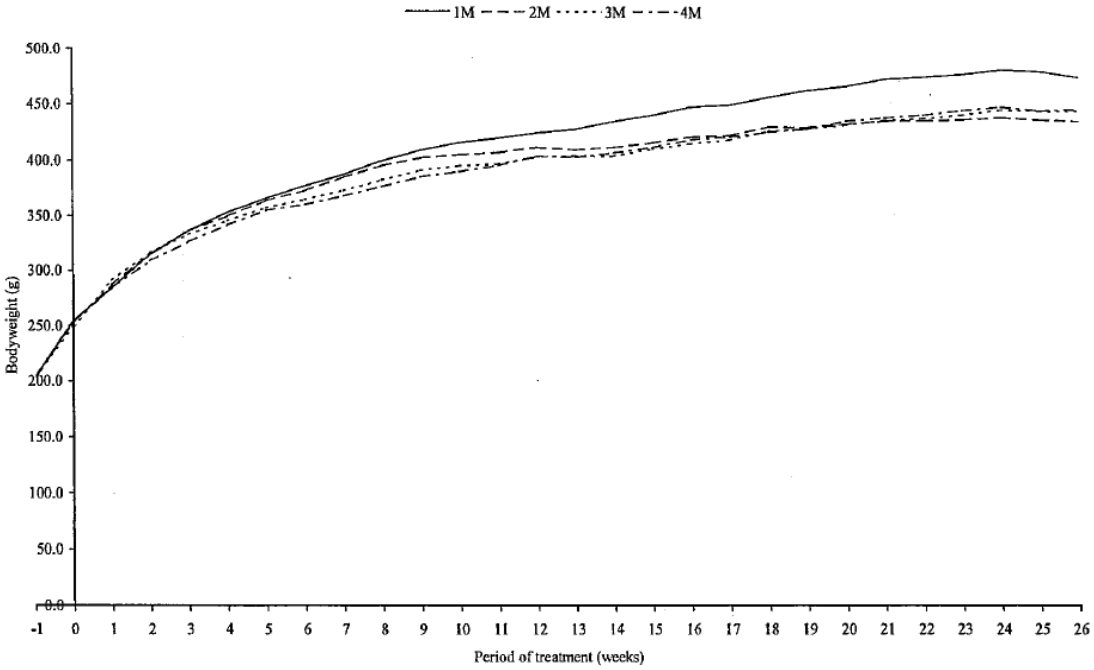
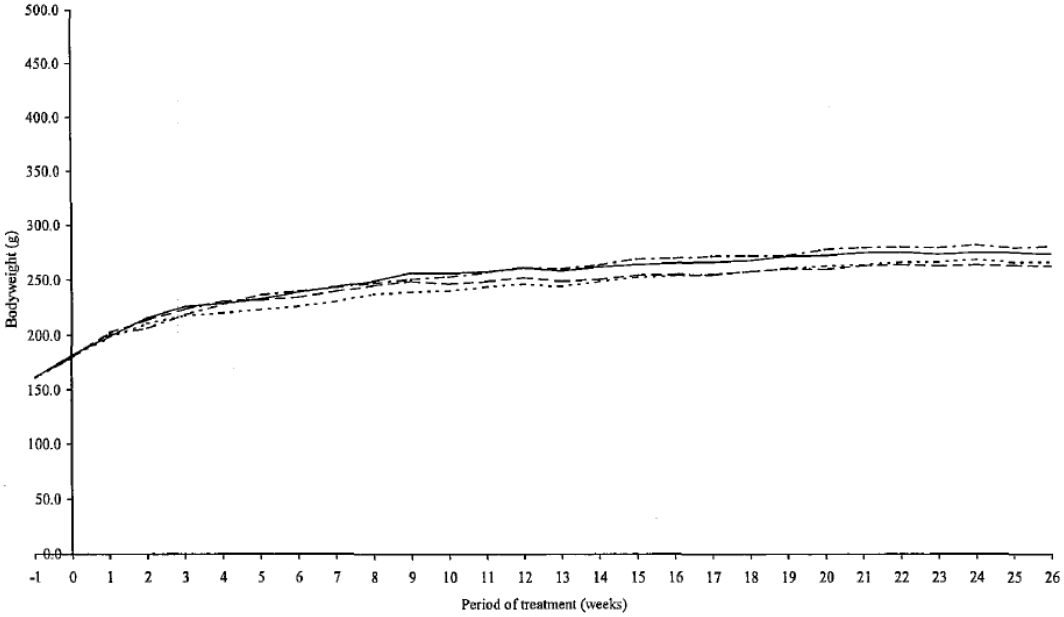


FIGURE 1 - continued

Bodyweight - group mean values versus period of treatment: females

Group	:	1	2	3	4
Compound	:	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	:	0	5	50	500



Food and Water Consumption [Weekly]
Food consumption was reduced 14% at week 1 in HDF; however, beginning week 4, HDF generally showed increased food consumption (~10%). No clear effect was observed in LDF, MDF, or treated males.

HD animals consumed more water (3-4x in males, ~3x in females) in weeks 13 and 27.

Ophthalmoscopy [Prior to study, Weeks 13 and 26]
All animals were examined by means of a Keeler all pupil indirect ophthalmoscope.

No drug-related findings were reported. One MDM showed anterior synechia of the iris.

Hematology [Weeks 13 & 25, sample taken before dosing after an overnight fast] & Coagulation [Week 27]
At Week 13, slight decreases (6-7%, [ss]) in RBC parameters were observed in HDF, but no clear effect was observed in males. Increases in reticulocyte (+9%, +12%) and platelet (+27%, +9%) counts were observed in HDF and HDM, respectively. At week 25, females showed a very slight, dose-related reduction (≤2-4%, [ss]) in RBC parameters; similar effects were not observed in males. Reticulocyte and platelet counts showed a slight, dose-related increase in females (up to ~20%), and HDM showed a 10% increase in platelets.

At Week 13, WBC counts showed changes, predominantly in females. Increases in neutrophils (~2x in MDF and HDF, however, the MDF average resulted from 2 outliers at 4-7x the control average whereas nearly all HDF animals were 2x the control average), monocytes (30-60%, MDF and HDF), and LUC (~2x in HDF) were observed. Reductions in lymphocyte (15-35%, treated females, d-r, [ss] at HD) and basophil (0 vs 0.1, HDF vs conF) counts were observed. In HDM, increases in absolute lymphocyte (11%), monocyte (33%), and LUC (22%) counts were also observed. At week 25, MDF and HDF showed increases in WBC counts (15-44%). Neutrophils (2-2.5x, MDF and HDF), monocytes (2x, HDF), LUC (1.5-2.3x, MDF and HDF), and lymphocytes (+15%, HDF) were increased in females. In HDM, increases in neutrophil (1.7x), basophil (1.5x), and LUC (18%) counts were observed (total WBC count +9%).

Coagulation parameters were dose-dependently affected in males at Week 27 (maximum increases of 19% in PT time and 15% for fibrinogen; maximum decrease of 4% in APTT time, all [ss]). In HDF, PT time and fibrinogen were increased (13% [ss] and 30% [ss], respectively), and APPT time was slightly decreased (-7%, [ss]).

Clinical Chemistry [Weeks 13 & 25, sample taken before dosing after an overnight fast]
Several clinical chemistry alterations were observed at both time points.

At Week 13, increased ALT (d-r, up to 1.8x in HDF; also 24% in HDM) was observed. However, dose-related reductions in ALP (≤36%) and AST (≤40%) were observed in females; and, AST was decreased in HDM by 23%. Total protein was increased (MDF,

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HDF and HDM, up to 19% in F and 4% in M), as were albumin (MDF and HDF up to 17%, HDM 5%) and globulin (MDF and HDF up to 23%). Triglycerides (d-r, up to 4.1x [ss] in HDF) and cholesterol (d-r, up to 2.7x [ss] in HDF) were also increased; in HDM, cholesterol was slightly increased (23%). Blood glucose was slightly increased in HDF (12%). BUN (-17%) and creatinine (-63%) were reduced in HDF. Generally, blood sodium and chloride were very slightly reduced (<5%), and calcium was slightly increased (5% in HDM, 17% in HDF).

At Week 25, most of the changes observed at Week 13 continued. ALT was increased (42% in HDF, 20% in HDM, [ss]). ALP (d-r, ≤24% in HDF) and AST (d-r, up to 43% in HDF and 34% in HDM) were reduced. Although total protein remained only very slightly increased in HDM (2%, [ss]), total protein (d-r, up to 12% in HDF [ss]), albumin (d-r, up to 6%, [ss]), and globulin (d-r, up to 26%, [ss]) were increased in females. The A/G ratio was slightly reduced by 15% in HDF [ss]. Cholesterol (d-r, as much as 2.3x in F [ss], and 26% in HDM [ss]), and triglycerides (as much as 3.1x in MDF and HDF [ss]) remained increased. BUN (-16% in HDF [ss]) and creatinine (as much as -29% in MDF and HDF, [ss]) continued to be reduced. Blood glucose was slightly increased (up to 10%) in MDF and HDF. Blood sodium and chloride remained very slightly reduced [ss], and calcium remained slightly increased (12% in HDF, and 3% in MDF and HDM [ss]).

Urinalysis [Weeks 13 & 27 (sic, 25), during 18 hr food deprivation, 1st 10 animals/sex/gp]

At Weeks 13 and 25, urine volume was increased in HD animals (3- 5x, [ss]). Nearly all HD animals showed increased volumes. Urine specific gravity was also slightly reduced (1-3%), reaching statistical significance in HDM in Week 25. No ketones were present in HDM, in comparison to other groups having at least 6 animals showing trace levels. In females, there was a dose-related increase in the presence of protein in the urine (see the sponsor's table, below). One HDF (#2410) showed RBC, large numbers of WBC, many bacteria, and a few crystals in the urine.

Summary of urinary protein results in females

Group/sex Dose (mg/kg/day)	1F 0	2F 5	3F 50	4F 500
Week 13				
None detected	6	4	4	1
Trace	1	2	1	2
1+	1	1	2	2
2+	0	0	2	2
3+	1	2	1	3
No animals examined	9	9	10	10
Week 26				
None detected	4	3	1	0
Trace	2	2	1	0
1+	6	7	10	2
2+	4	4	0	4
3+	3	3	8	14
No animals examined	19	19	20	20

Significant when compared with Group 1: a – p<0.05 (Fisher's Exact Test)

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Gross Pathology
Animals were sacrificed by carbon dioxide asphyxiation and subsequent exsanguination.

Few organs showed macroscopic alterations. See excerpts from the sponsor's summary Table 9, below. Enlarged liver was the single clear finding in all HD animals. Stomach (in HDM), uterus, and mammary gland also showed gross alterations. Masses were observed in 1 MDF (proximal area of vagina) and one HDF (inguinal region of skin).

TABLE 9

Macropathology - group distribution of findings for animals killed after 26 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	:	0	5	50	500

Tissue and finding	Group/sex:	1M	2M	3M	4M	1F	2F	3F	4F
	Number Examined:	19	20	20	20	20	20	20	20
Liver									
Dark median lobe		0	0	1	0	0	0	0	0
White caudate lobe		0	0	0	1	0	0	0	0
White left lobe		1	0	0	0	0	0	0	0
Size increased		0	0	0	20**	0	0	0	20**
Kidney									
Cyst		0	0	0	0	0	0	0	1
Dark discolouration		0	1	3	0	0	0	0	0
White discolouration		0	0	0	0	0	0	0	1
Stomach									
Red glandular area		0	0	0	3	1	2	0	0
White glandular area		0	0	0	1	0	0	0	0
Raised cardia area		0	1	0	0	0	0	0	0
Uterus									
Abnormal contents		-	-	-	-	1	2	1	0
Dilatation		-	-	-	-	3	8	6	7
Dark, midhorn		-	-	-	-	0	0	1	0
Ovary									
Cyst		-	-	-	-	1	1	0	2
Mammary gland									
Galactocoele		0	0	0	0	0	0	0	2

Organ Weights [See the sponsor's list, below]

Adrenals	Pituitary
Brain	Prostate with seminal vesicles
Heart	Spleen
Kidneys	Testes
Liver	Thyroid
Ovaries	Uterus with cervix

Several organs demonstrated changes in weight. See the sponsor's summary tables, below. Heart, liver, adrenal gland, and kidney weights were increased in both males

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and females. In females, ovary and spleen weights were also increased. Pituitary gland in both sexes and prostate in males showed decreased organ weights.

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Organ weights - group mean values relative to terminal bodyweight (g/g terminal bodyweight x1000) for animals killed after 26 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	:	0	5	50	500

Group /sex		Heart	Liver	Adrenal glands	Pituitary gland	Thyroid gland	Testes	Prostate	Spleen	Brain	Kidneys
1M	Mean	3.415	27.896	0.129	0.024	0.043	9.016	6.387	1.789	4.392	7.314
	SD	0.223	1.689	0.014	0.004	0.010	0.900	0.720	0.163	0.292	0.451
	N	19	19	19	19	19	19	19	19	19	19
2M	Mean	3.445	28.547	0.138*	0.023	0.045	9.667*	5.767*	1.870	4.715**	7.641
	SD	0.241	2.357	0.015	0.004	0.012	0.823	1.126	0.193	0.321	0.542
	N	20	20	20	20	20	20	20	20	20	20
3M	Mean	3.416	30.789**	0.132	0.023	0.047	9.975**	6.206	1.818	4.596*	7.536
	SD	0.293	1.820	0.014	0.004	0.008	0.857	0.912	0.164	0.290	0.452
	N	20	20	20	20	20	20	20	20	20	20
4M	Mean	3.680**	46.623**	0.143**	0.021*	0.043	10.000**	5.434**	1.888	4.516	8.396**
	SD	0.240	3.414	0.014	0.004	0.012	0.898	0.854	0.190	0.312	0.598
	N	20	20	20	20	20	20	20	20	20	20

* = $p<0.05$, ** = $p<0.01$ for comparison versus the Control using Dunnett's test

Organ weights - group mean values relative to terminal bodyweight (g/g terminal bodyweight x1000) for animals killed after 26 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	:	0	5	50	500

Group /sex		Heart	Liver	Adrenal glands	Pituitary gland	Thyroid gland	Ovaries	Uterus	Spleen	Brain	Kidneys
1F	Mean	3.923	26.405	0.257	0.059	0.065	0.321	3.537	2.161	7.067	7.255
	SD	0.308	1.559	0.043	0.012	0.018	0.088	0.924	0.195	0.469	0.515
	N	20	20	20	20	20	20	20	20	20	20
2F	Mean	3.983	27.499	0.276	0.051*	0.074	0.297	3.816	2.167	7.318	7.716**
	SD	0.373	2.047	0.052	0.008	0.018	0.075	0.877	0.183	0.455	0.487
	N	20	20	20	20	20	20	20	20	20	20
3F	Mean	4.127	31.358**	0.267	0.051*	0.070	0.313	3.764	2.292	7.133	7.973**
	SD	0.344	2.596	0.029	0.009	0.025	0.085	0.852	0.253	0.541	0.421
	N	20	20	20	20	20	20	20	20	20	20
4F	Mean	4.333**	53.233**	0.282	0.052*	0.074	0.372	3.642	2.318*	6.820	9.057**
	SD	0.287	4.002	0.040	0.013	0.021	0.110	1.313	0.285	0.502	0.693
	N	20	20	20	20	20	20	20	20	20	20

* = $p<0.05$, ** = $p<0.01$ for comparison versus the Control using Dunnett's test

Histopathology [See list at end of general tox section; 4 μm, H&E stained]

Adequate Battery Yes

Peer Review Report states yes, but no documentation provided

Study Pathologist (b) (4)

NOTE: A separate, signed pathologist's report was not provided in the report; a signed pathology report was provided upon request (N 205-677, SDN11).

Histological Findings

All tissues from control and HD animals were examined, as well as all early mortalities. Tissues reported as grossly abnormal were examined for all animals. Also, the target tissues were examined in all animals, including: kidneys, liver, and spleen. An additional section of kidney from each male rat was stained by Mallory-Heidenhain method. Other special stains used on the liver and/or kidneys to further specify drug-related changes included Oil Red O, Gomori modified iron, periodic acid-Schiff (PAS), and Hall's and Kinyoun carbol fuchsin.

Liver, kidney, and spleen were identified as clear target organs. See the sponsor's summary table of selected liver findings with severity, below. According to the sponsor, the centrilobular hypertrophy observed in the liver was presumed to reflect "an adaptive response" related to induction of microsomal enzymes (and possibly contributing to the changes in drug exposure over time, as well as the appearance of increased organ weight and gross enlargement), but the focal necrosis clearly indicated a "toxic" insult. A complete list of the histological liver findings is excerpted from the sponsor's summary data, below. Many of the clinical chemistry alterations (e.g., enzymes, glucose, triglyceride, cholesterol, protein) may reflect the "adaptive" and/or "toxic" changes.

Treatment related findings in the liver after 26 weeks of treatment

Group/sex Dose (mg/kg/day)	1M 0	2M 5	3M 50	4M 500	1F 0	2F 5	3F 50	4F 500
Centrilobular hepatocyte hypertrophy								
Minimal	0	0	9	1	0	0	7	0
Slight	0	0	0	12	0	0	7	0
Moderate	0	0	0	7	0	0	0	20
Total	0	0	9a	20a	0	0	14a	20a
Focal hepatocyte necrosis								
Minimal	0	0	0	1	0	0	1	3
Slight	0	0	0	3	0	0	0	0
Total	0	0	0	4	0	0	1	3
Number of animals examined	19	20	20	20	20	20	20	20

Significant when compared with Group 1: a – $p<0.05$ (Fisher's Exact Test)

Histopathology - group distribution of findings for animals killed after 26 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	:	0	5	50	500

Tissue and Finding	Group/Sex: Number:	1M 19	2M 20	3M 20	4M 20	1F 20	2F 20	3F 20	4F 20
Liver	Number Examined:	19	20	20	20	20	20	20	20
Clear Cell Foci		0	0	0	0	1	0	0	0
Eosinophilic Foci		0	0	0	2	0	0	0	0
Hepatocyte Hypertrophy, Centrilobular		0	0	9	20	0	0	14	20
Hepatocyte Necrosis, Focal		0	0	0	4	0	0	1	3
Hepatocyte Vacuolation, Focal		0	0	0	0	1	0	0	0
Inflammation, Focal		7	10	10	10	6	3	3	8
Pigment in Hepatocytes		0	0	0	1	0	0	0	0

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In the kidneys, the sponsor related the majority of the alterations to exacerbated chronic progressive nephropathy (CPN). See the sponsor's summary tables of kidney findings and selected findings with severity, below. It is of importance that HDF showed a higher incidence of more severe CPN. This is not the usual pattern for the finding; males are typically more affected. Kidneys showed increased organ weights. Clinical chemistry (e.g., BUN, creatinine, and electrolytes), hematological (e.g., WBC), and urinalysis (e.g., increased volume, decreased specific gravity, and proteinuria in HDF) alterations were believed to reflect this change.

Treatment related findings in the kidneys after 26 weeks of treatment

Group/sex	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Dose (mg/kg/day)	**0**	**5**	**50**	**500**	**0**	**5**	**50**	**500**
Chronic progressive nephropathy								
Minimal	14	13	17	5	9	4	7	4
Slight	2	4	2	13	0	2	4	12
Moderate	1	1	0	2	0	0	0	4
Total	17	18	19	20	9	6	11	20a
Cortical tubules with hyaline droplets								
Minimal	17	13	17	7	4	2	11	17
Slight	1	1	0	13	0	0	0	3
Total	18	14	17	20	4	2	11a	20a
Interstitial pigmented macrophages								
Minimal	6	8	14	13	2	2	13	14
Slight	0	1	0	1	0	0	0	3
Total	6	9	14a	14a	2	2	13a	17a
Cortical tubular pigment								
Minimal	6	9	14	16	0	2	9	15
Slight	1	1	0	2	0	0	0	2
Total	7	10	14	18a	0	2	9a	17a
Cortical tubular dilatation								
Slight	0	0	0	0	0	0	0	3
Total	0	0	0	0	0	0	0	3
Number of animals examined	19	20	20	20	20	20	20	20

Significant when compared with Group 1: a – $p<0.05$ (Fisher's Exact Test)

Histopathology - group distribution of findings for animals killed after 26 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	:	0	5	50	500

Tissue and Finding	Group/Sex: Number:	1M 19	2M 20	3M 20	4M 20	1F 20	2F 20	3F 20	4F 20
Kidneys	Number Examined:	19	20	20	20	20	20	20	20
Chronic Progressive Nephropathy		17	18	19	20	9	6	11	20
Cortical Cyst(s)		0	0	0	0	0	0	0	1
Cortical Scarring		1	0	0	1	1	0	0	1
Cortical Tubular Basophilia		1	0	0	0	0	1	0	0
Cortical Tubular Dilatation		0	0	0	0	0	0	0	3
Cortical Tubular Pigment		7	10	14	18	0	2	9	17
Cortical Tubules with Hyaline Droplets		18	14	17	20	4	2	11	20
Hyperplasia, Pelvic Epithelium		0	0	0	0	0	1	0	0
Inflammatory Infiltration - Pelvic Epithelium		0	0	0	0	0	1	0	0
Interstitial Inflammation		0	1	0	0	0	0	1	2
Interstitial Pigmented Macrophages		6	9	14	14	2	2	13	17
Medullary Cyst(s)		0	0	0	1	0	0	0	0
Mineralisation, Papillary/Pelvic Epithelium		0	0	1	0	0	0	0	1
Pelvis, Inflammation		0	0	0	0	0	1	0	0
Stromal Fatty Infiltration		0	0	0	0	0	0	1	0
Tubular Casts		1	1	1	0	4	8	5	0
Tubular Hyperplasia, Simple		0	1	0	0	0	0	0	1

Hemosiderosis and extramedullary hematopoiesis were observed in spleen. The hemosiderosis was thought to reflect an increased rate of removal of RBCs from peripheral blood. The sponsor considered the splenic extramedullary hematopoiesis (and reticulocytosis) a "compensatory response." The sponsor further posited that the female prevalence reflected the higher exposures in females.

Treatment related findings in the spleen after 26 weeks of treatment

Group/sex Dose (mg/kg/day)		1F 0	2F 5	3F 50	4F 500
Haemosiderosis					
	Minimal	6	4	8	6
	Slight	0	1	4	13
	Total	6	5	12	19a
Increased extramedullary haemopoiesis					
	Minimal	1	2	4	2
	Slight	0	0	3	10
	Total	1	2	7a	12a
Number of animals examined		20	20	20	20

Significant when compared with Group 1: a – $p<0.05$ (Fisher's Exact Test)

Other organs also showed histologic changes. The stomach, lymph nodes (cervical, mesenteric), mammary gland, and female reproductive organs showed changes. See excerpts from the sponsor's summary Table 10, below. In addition to the noted changes, a few low incidence alterations of unclear toxicologic significance were observed. Focal pituitary hyperplasia of the pars distalis was observed in 1 HDM and 1 HDF. Changes in thyroid (called "prominent ultimobranchial cysts" by the pathologist)

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were observed in 3 HDM and 7 HDF (compared to 1 control of each sex). A recent paper may shed light on these structures, noted to change appearance as rats age (cf., Vezquez-Roman et al., 2013) The description suggests that these may represent forms that generally occur in older rats (predominantly female; "cystadenomata" forms at ≥ 18 months of age); the reason for and importance of the apparent early presence is unclear. Although ultimobranchial cysts are generally disregarded as embryological remnants without toxicologic import, there is a possibility that the observed pituitary and thyroid changes (cf. Chrisov et al., 1973; Conde et al., 1992; Martin-Lacave et al., 1992) reflect changes accompanying the observed centrilobular hypertrophy of the liver, exhibiting a known pattern of hormonal axis dysregulation in rodents. The gross observation of a vaginal mass was described histologically as a squamous epithelial cyst. Although heart weight was altered, there was no clear histopathological correlate.

Group	:	1	2	3	4
Compound	:	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	:	0	5	50	500

Tissue and Finding	Group/Sex: Number:	1M 19	2M 20	3M 20	4M 20	1F 20	2F 20	3F 20	4F 20
Stomach	Number Examined:	19	1	0	20	20	2	0	20
Dilated Glands		14	0	0	10	18	1	0	12
Epithelial Hyperplasia - Nonglandular Region		0	0	0	1	0	0	0	0
Mucosal Erosion - Glandular Region		1	0	0	3	1	2	0	0
Mucosal Necrosis - Glandular Region		0	0	0	1	0	0	0	0
Submucosal Oedema - Glandular Region		0	0	0	1	0	2	0	0
Submucosal Oedema - Nonglandular Region		0	0	0	2	1	0	0	0
LN Cervical	Number Examined:	19	0	0	19	20	0	0	19
Plasmacytosis		3	0	0	4	0	0	0	1
LN Mesenteric	Number Examined:	19	0	0	20	20	0	0	20
Paracortex - Increased Cellularity		0	0	0	1	0	0	0	0
Sinus Erythrocytosis/Erythrophagocytosis		2	0	0	2	0	0	0	2
Sinus Histiocytosis		2	0	0	0	5	0	0	7
Mammary	Number Examined:	19	0	0	20	20	0	0	20
Galactocele(s)		0	0	0	0	0	0	0	2
Secretory Activity		0	0	0	0	0	0	0	3
Ovaries	Number Examined:	-	-	-	-	20	1	0	20
Follicular Cyst(s)		-	-	-	-	1	1	0	4
Uterus	Number Examined:	-	-	-	-	20	8	6	20
Luminal Dilatation		-	-	-	-	6	8	6	14
Uterine Cervix	Number Examined:	-	-	-	-	20	0	0	20
Epithelial Keratinisation		-	-	-	-	13	0	0	16
Heart	Number Examined:	19	0	0	20	20	0	0	20
Arteritis/Periarteritis		0	0	0	1	0	0	0	0
Myocardial Fibrosis		0	0	0	1	0	0	0	0

Toxicokinetics [See the sponsor's tables, below] Rpt MAP005/214778; 920002144

Occasion	Group and sex	Time of sampling (hours after dosing) 1	2	4	8	12	24
		Animal numbers					
Day 1	2M	1201-1203	1204-1206	1207-1209	1210-1212	1213-1215	1216-1218
	2F	2201-2203	2204-2206	2207-2209	2210-2212	2213-2215	2216-2218
	3M	1301-1303	1304-1306	1307-1309	1310-1312	1313-1315	1316-1318
	3F	2301-2303	2304-2306	2307-2309	2310-2312	2313-2315	2316-2318
	4M	1401-1403	1404-1406	1407-1409	1410-1412	1413-1415	1416-1418
	4F	2401-2403	2404-2406	2408,2409, 2419*	2410-2412	2413-2415	2416-2418

* - 4F 2407 replaced with 4F 2419 due to signs observed on Day 1

Occasions	Group and sex	Time of sampling (hours after dosing) 1	2	4	8	12	24
		Animal numbers					
Weeks 14 & 26	2M	1201-1203	1204-1206	1207-1209	1210-1212	1213-1215	1216-1218
	2F	2201-2203	2204-2206	2207-2209	2210-2212	2213-2215	2216-2218
	3M	1301-1303	1304-1306	1307-1309	1310-1312	1313-1315	1316-1318
	3F	2301-2303	2304-2306	2307-2309	2310-2312	2313-2315	2316-2318
	4M	1401-1403	1404-1406	1407-1409	1410-1412	1413-1415	1416-1418
	4F	2401-2403	2404-2406	2408,2409, 2419	2410-2412	2413-2415	2417, 2418, 2421#

- 4F 2416 killed on Day 3, replaced with 4F 2421

Blood samples were collected from the first 18 controls of each sex but were discarded without analysis. Systemic exposure to tasimelteon (BMS-214778) was dose-related. C_{max} generally showed greater than dose-proportional increases on day 1 and less than dose-proportional increases at weeks 14 and 26 (see the sponsor's table below); AUC generally showed greater than dose-proportional increases on day 1 and approximately dose-proportional increases at weeks 14 and 26. Exposure was higher (2-5x) in females than in males. The sponsor indicated there was some accumulation at LD. The sponsor provided the following summary table for TK parameters.

Relationship between dose and C_{max} and AUC_{24} for BMS-214778

Study Week	C_{max} ratio Male	C_{max} ratio Female	$AUC_{(0-t)}$ ratio Male	$AUC_{(0-t)}$ ratio Female
Day 1	1:70:470	1:41:220	1:50:576	1:51:510
14	1:10:44	1:15:15	1:10:118	1:25:146
26	1:9:25	1:13:21	1:13:105	1:23:108

C_{max} and AUC_{24} for BMS-214778

Dose (mg/kg/day)	Study Week	C_{max} (ng/mL) Male	C_{max} (ng/mL) Female	$AUC_{(0-t)}$ (ng.h/mL) Male	$AUC_{(0-t)}$ (ng.h/mL) Female
5	Day 1	39	178	249	595
	14	162	661	481	1114
	26	304	805	670	1458
50	Day 1	2722	7247	12439	30326
	14	1640	10127	4912	27496
	26	2603	10053	8435	34176
500	Day 1	18333	39196	143301	303457
	14	7060	10141	56494	162891
	26	7577	16761	70085	158164

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Interspecies Parent and Metabolite Comparison

For reference, the sponsor also provided the following assessment of the estimated metabolite exposures in rats in the 6-month toxicity study (Table 8 from the Nonclinical Toxicology Written Summary, below). It is noted that the sponsor used 363 ng·hr/ml as the human exposure at the RHD (rather than the summary value of 411 ng·hr/ml across studies), and the Week 26 AUC exposures (i.e., the highest exposures after Day 1 AUC exposures) in male and females rats.

Table 8: Tasimelteon metabolite levels in rats estimated from measured tasimelteon plasma levels in Study TAJ0007 (Week 26) using the metabolite/tasimelteon AUC ratios determined in Study TAJ0014

Dose		AUC_{0-24} (ng.h/mL)								
Rat		Tasimelteon	M9 ratio[a]	M9	M12 ratio[a]	M12	M13 ratio[a]	M13	M3 ratio[a]	M3
50[b] mg/kg	M	8,435	0.30	2,531	2.40	20,244	0.03	253	0.78	6,579
	F	34,176	0.14	4,785	2.60	88,858	0.02	684	0.39	13,329
500 mg/kg	M	70,085	0.27	18,923	1.92	134,563	0.06	4,205	0.75	52,564
	F	158,164	0.16	25,306	1.70	268,879	0.04	6,327	0.67	105,970
Human[c] 20 mg QD		363	--	423	--	619	--	353	--	192

[a] Metabolite/tasimelteon AUC ratios from Study TAJ0014 at comparable doses (corrected for molecular weights)
[b] Metabolite/tasimelteon AUC ratios for 50 mg/kg averaged from values for 25 and 100 mg/kg doses from Study TAJ0014
[c] Data for tasimelteon and M9, M12, and M13 are from Study VP-VEC-162-1110: An Open-Label, Single Sequence Study to Assess the Effect of Multiple Doses of Tasimelteon on the Cytochrome P450 3A4 and 2C8 Enzymes using Midazolam and Rosiglitazone as Substrates. Data for M3 are from group 2 in Study VP-VEC-162-1107: An open-label, single-dose, parallel group study to assess the effects of smoking status, age and body size on the pharmacokinetics, safety, and tolerability of tasimelteon in healthy volunteers.

Study Title: BMS-214778: ONE-YEAR ORAL TOXICITY STUDY IN MONKEYS

Study no.:	057-005 (b) (4); #97331 (BMS)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/24/98
GLP compliance:	Yes, except: CoA for the test article, Test article sample analysis, Bulk test article stability, missing QA statements for the TK data and report, and Stability of the formulated test article (previously determined in Bristol-Myers Squibb Study No. 97331) is not included in this report
QA statement:	Yes (b) (4) Pathology QA (b) (4)
Drug, lot #, and % purity:	BMS-214778-01, lot N031C-214778-01, purity 99.3%

Methods (see the sponsor's summary table, below)

Frequency of dosing:	Daily
Route of administration:	PO, nasogastric intubation or oral gavage
Dose volume:	1 mL/kg
Formulation/Vehicle:	Polyethylene Glycol-400 (PEG-400), lot M18621 and N01641
Species/Strain:	adult male and female Cynomolgus monkeys (b) (4)
Age:	Not provided
Weight:	M: 2.09 to 3.86 kg and F: 1.93 to 3.62 kg
Dosed fasted or fed:	Not specified
Unique study design:	Interim sacrifice: see sponsor's table below
Deviation from study protocol:	The study director stated that three deviations occurred, none of which affected the integrity of the study.

Group Number	Dosage Level (mg BMS-214778/kg)	Dose Volume mL/kg	Concentration (mg BMS-214778/mL)	Study Animals* Males	Study Animals* Females
1	Vehicle Control	1	0	7	7
2	3	1	3	7	7
3	20	1	20	7	7
4	150	1	150	7	7

*Up to three males and three females per group were necropsied after six months of vehicle or test material treatment. The remaining animals were necropsied after at least one year of vehicle or test material treatment and were dosed through the last day prior to necropsy.

Observations and Results

Dosing Solution Analysis

Drug formulations were prepared one week prior to use. Duplicate samples were taken from the dosing formulations at Weeks 1,10, 27, 39, and 52. One sample of each formulation concentration was analyzed. Measured BMS-214778 concentration was within 6% of nominal in all samples.

The sponsor stated that the stability of BMS-214778 formulations at the concentrations to be used in this study was previously established for seven days at room temperature in a previous study (i.e., Bristol-Myers Squibb Study No. 97331).

Mortality [2x daily]

No drug-related mortalities were reported; however, four animals died on study. Two males were found dead during the first week of the study and were replaced. MDM #169 and HDF #178 were found dead after approximately four and seven months of dosing, respectively. According to the sponsor, the three males each had acute fibrinous inflammation in the lungs and the female was remarkable for lung hemorrhage; these findings were consistent with accidental deaths.

In addition to the findings related to the cause of death, a few other histopathologic findings were observed in these animals. Hemorrhage or vacuolation in the white matter of the brain and/or gray matter of the spinal cord was observed in the HDM and HDF. In the liver, mild congestion was observed in the MDM and HDM, and minimal chronic periportal inflammation was observed in all four early mortalities. In the kidney, minimal mineralization of the papilla was observed in the HDM and the HDF; chronic interstitial inflammation was also observed in the HDF. Mild atrophy of the thymus was observed in the HDM. In the HDF, changes were also observed in the aorta (minimal fibrous plaque), stomach (bacteria, ulceration, and lymphocyte accumulation), ovary (minimal mineralization), vagina (mild chronic inflammation), lachrymal gland (minimal chronic inflammation), and pineal gland (minimal mineralization).

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Clinical Signs [2x daily, prior to and after dosing; detailed physical exams pre-test, Week 13, Week 26, Week 39, and Week 52]

Convulsions were observed in 2 HD monkeys (M #183 on D34 and F #174 on D163). These animals also showed flaccidity (M only), salivation, gasping (M only), and labored breathing in association with the convulsions. (The HDM was sacrificed at the interim assessment.)

According to the sponsor, drug-related "decreased appetite" was observed at ≥ MD (11/1, 10/2, 19/5, and190/6 in conM, LDM, MDM, and HDM, respectively, and 107/7, 68/6, 173/6, and 183/6 in conF, LDF, MDF, and HDF, respectively [# observations / # animals]). Occasional excessive salivation, emesis (F), and alopecia (head, limbs) was observed at ≥ MD. Decreased activity was demonstrated in most HD animals beginning after 6 weeks and continuing throughout the study.

Of unclear relationship to drug (although the sponsor attributed to drug) are findings in one LDM (#143). This animal showed an episode of coldness, prostration, decreased motor activity, and ataxia on D177; the ataxia and decreased motor activity continued through D178 and D179, respectively. Veterinary care records indicated that this animal was "weak, unable to sit up" and showed "slight dehydration, pale mucous membranes" on D177; a 5% dextrose solution was administered SC. This animal's physical examination record in week 26 also showed thinness, paleness, a low body temperature (95.3 vs. 98-100° C in controls), and a prolonged capillary refill time of < 3s (vs. < 1s in most other animals).

Body Weights [Predose on D1, weekly, last dosing day, and prior to necropsy]

Slightly reduced (~5%) average body weights were observed for HD animals during the first 6 months of the study. During the last few months of the study, the LD animals tended to show increased average body weights (up to 15% in M and 10% in F). See the sponsor's Figures 1 and 2, below.

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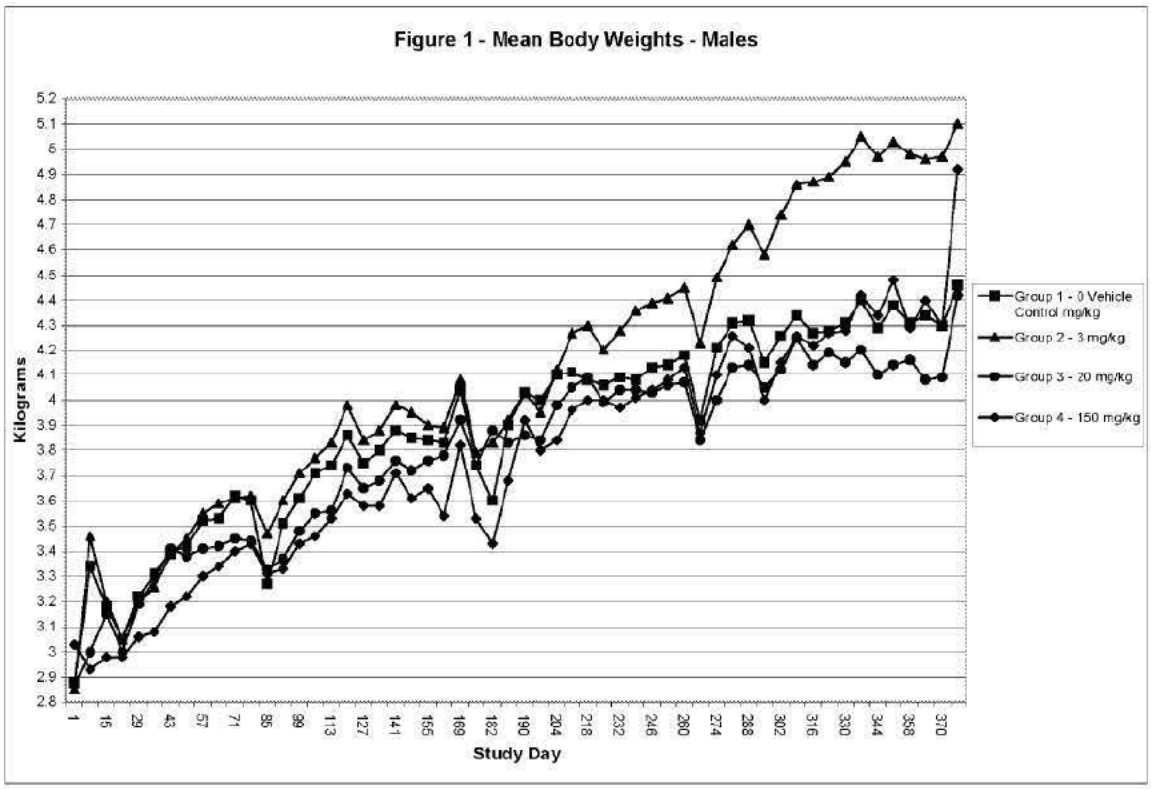

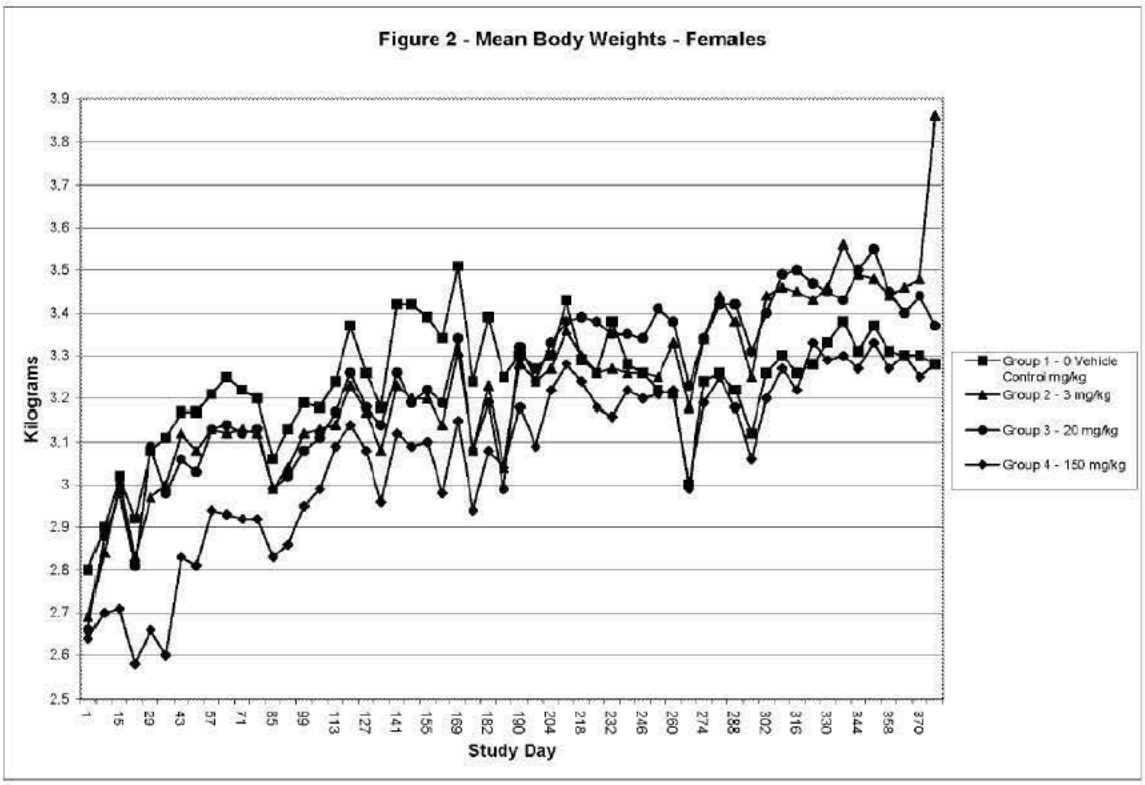

Food Consumption [Daily, by visual assessment]
Summary data were not presented. The sponsor indicated that "any depression in appetite was noted in the study file"; this appears to have been reported under **Clinical Signs**, and qualitative measurements were not provided.

Ophthalmoscopy [Pre-test, Week 13, Week 26, Week 39, and Week 52]
An ophthalmological examination using a slit lamp was conducted (under ketamine anesthesia) on all available animals by a Board Certified Veterinary Ophthalmologist, (b) (4)

No drug-related alterations were reported.

ECG [Pre-test, Week 13, Week 26, Week 39, and Week 53]
Electrocardiogram tracings were taken from all animals using six leads (I, II, III, aVR, aVL, and aVF); no anesthesia was used. The tracings were interpreted by (b) (4) and a written report was provided; some tracings were also sent to (b) (4) but no report was provided.

The veterinary cardiologist did not report drug-related changes in heart rate, heart rhythm, or ECG parameters (including QTcF) at any dose. Altered blood pressure was not reported. However, blood pressures in HDF (systolic, diastolic, MAPR) were slightly increased compared to controls (10-30%) on D269 and D365. In the LDM (#143) sacrificed early (at interim), the Week 26 HR and QTc also appeared slightly increased.

Hematology [Pre-test, Week 13, Week 26, Week 39, and Week 52]
Food and water were withheld overnight prior to collection, except for Weeks 13 and 52. The blood samples were evaluated for the following parameters: red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, red blood cell morphology, platelet count, white blood cell count, lymphocyte count, monocyte count, eosinophil count, basophil count, fibrinogen, activated partial thromboplastin time, prothrombin time, and other cells (as appropriate). The clinical pathology data were interpreted by (b) (4)

The sponsor reported slightly (<15%) reduced RBC parameters (i.e., hematocrit, hemoglobin concentration, and RBC count) at HD; the reduction was observed at Week 13 and throughout the study (to Week 39 in HDF). Eosinophils were increased (4x and 2.75x at weeks 39 and 52) in HDF. In the LDM (#143 sacrificed early (at interim), hematology assessment at W26 showed reductions in WBC (63%), RBC (29%), hemoglobin (35%), hematocrit (33%), and increased fibrinogen (20%), compared to conM.

A few other changes of unclear toxicologic significance were observed at individual measurement times. Some changes appeared most severe at Week 13 but appeared to resolve. Platelets were increased (28% in HDM only at Week 13; in HDF, 90% at Week 13, 30% at Week 26, and 40% at Week 39). Fibrinogen was reduced as much

as 28% in HDM (appeared dose-related) from Week 26 on; reductions were also seen in HDF from Week 13 on (10-35%), with some reduction in MDF as well. Prothrombin time was very slightly reduced at Week 13 (HDM, 8%) and Week 26 (MDM and HDM, 7%). APPT was increased ~26% in HDM only at Week 26.

Clinical Chemistry [Pre-test, Week 13, Week 26, Week 39, and Week 52]
Food and water were withheld overnight prior to blood collection, except for Weeks 13 and 52. The samples were processed and evaluated for the following parameters: total protein, albumin, globulin, albumin/globulin ratio, glucose, cholesterol, triglycerides, total bilirubin, urea nitrogen, creatinine, creatine kinase, alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), calcium, phosphorus, sodium, potassium, and chloride.

The sponsor reported slightly increased serum ALT (2-3x that of ConM, highest at Week 13, with some increase in MDM; up to 2x that of ConF, from Week 26 on) and GGT (30-50% in HDM, up to 70% in HDF); the sponsor indicated that these findings correlated with increased liver weight at necropsy. According to the sponsor, these increases were generally seen at Week 13 and, although they fluctuated, were usually seen throughout the study. Additionally, HD animals showed increased serum triglycerides (~2x ConM at Week 52 in HDM only) and reduced ALP (as much as 30-40%). In the LDM #143 sacrificed early (at interim), LFTs were increased 3-5x, and albumin and phosphorus were decreased (32% and 73%, respectively, compared to ConM) at Week 26.

In addition to the changes noted by the sponsor, a few additional parameters showed changes of unclear toxicologic significance at individual measurement times. Some changes appeared greater at Week 13 than Week 26. Serum glucose was increased (20-25%) in HD animals at Week 13 only. Serum albumin was slightly increased (14%) in HD at Weeks 13 and 26. Serum total bilirubin was reduced as much as 75% beginning Week 13 in HDM; reductions (50-70%) occurred in HDF at Weeks 39 and 52. Creatine kinase values were extremely variable, but were reduced 40% in HDM at Week 52. Serum calcium was increased 6-10% in HDM. Serum potassium was increased 16% in HDF at Week 13 only.

Urinalysis [Pre-test, Week 13, Week 26, Week 39, and Week 52]
Food and water were withheld overnight prior to collection, except for weeks 13 and 52. The urine samples were evaluated for the following parameters: pH, protein, glucose, ketone, bilirubin, urobilinogen, leukocytes, specific gravity, color, clarity, occult blood, nitrite, and microscopic examination of urine sediment.

No drug-related changes were reported. Ketones were occasionally increased at HD.

Gross Pathology [D183/184 or D370/371]
No summary table was provided. The sponsor reported enlarged liver in one HDM (#187) after one year of dosing, which correlated with increased liver weight. In addition to this, a few other findings noted in liver (cysts, foci, or pigment/mottled) were without

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corresponding histology or were histologically observed as dilation of bile ducts (1 LDF), tension lipidosis (1 LDF), chronic inflammation (MDF), serosal/subserosal fibrosis (2HDF), or congestion (1 HDF).

Although few organs showed a clear drug-related effect, there were a few changes noted in stomach, intestines, and/or lymph nodes (these appear to mostly be related to parasites), spleen, kidney, and ovary. White focus(i) in the spleen was observed in one LDF and one HDF; this correlated histologically to serosal fibrosis. One HDF showed a dark focus in the kidney that was histologically described as congestion. Gross observations in the ovary (i.e., cysts, focus, nodule) were not associated with microscopic changes.

Organ Weights
Organ weighs were assessed prior to fixation except for pituitary gland of #159.

The sponsor reported increased liver weights at HD at 6 months and one year (~1.8x that of ConM [ss; d-r] and ~1.4x that of ConF); the increase was reported to correlate with reduced total systemic exposure and slightly increased serum ALT and GGT.

Relative thyroid gland weight was increased up to 40% in MDM and HDM at 1 year (dose-related) but was slightly reduced in MDF and HDF (~20%).

Histopathology

Adequate Battery Yes, with a few omissions (see Appx. 2)

Peer Review Yes, but limited

(b) (4)

See sponsor's summary table, below

The histology was reviewed by the peer review pathologist from the following animals:

Group	Dose (mg/kg/day)	Male ID	Tissues	Female ID	Tissue
1	0	129, 133	All	130, 132	All
1	0	131, 135	Liver	--	--
1	0	--	--	134, 136	Uterus, ovary
2	3	145, 147, 149 185	Liver	--	--
2	3	--	--	144, 146, 148, 150	Uterus, ovary
3	20	157,159 161,163	Liver	--	--
3	20	--	--	158, 160, 162, 164	Uterus, ovary
4	150	173, 177	All	172, 176	All
4	150	171, 187	Liver	--	--
4	150	--	--	174, 178	Uterus, ovary

Separate, Signed Pathology Report **Yes (revised narrative)**

(b) (4)

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Histopathology was performed on all tissues from all animals and all gross lesions. Fixed tissues were shipped to (b) (4) for trimming, embedding, and sectioning. Slides were stained with hematoxylin and eosin.

Histological Findings

The sponsor reported no drug-related histopathologic findings and noted that "parasites were observed frequently in tissue sections." The sponsor stated that the observed hypospermatogenesis in testis and oligospermia in epididymis (notably in LDM #143, showing the relatively severe clinical signs and sacrificed early at interim) were interpreted by the pathologist as sexual immaturity; other histopathological findings in this animal included animal chronic periportal inflammation in the liver, moderate, diffuse follicle dilatation in the thyroid, minimal chronic interstitial inflammation in the kidney, minimal chronic inflammation in the meninges of the forebrain, and minimal chromatolysis of the lumbar spinal cord.

Noted in the report but not in the summary data tables was a "lack of corpora lutea" in the three remaining HDF (# 174, 176, and 178) at one year, which the pathologist stated "indicates a lack of recent estrus cycling." The sponsor stated that relationship to drug could not be excluded, but that it is "… not unusual to see monkey ovary sections that do not contain corpora lutea." Melatonin is known to have endocrine effects (and effects on estrus cycling were observed in rats); therefore, this finding is considered adverse by the reviewer.

A few changes (increased incidence and/or increased severity) of unclear toxicologic importance were observed, at both interim and terminal sacrifice. Overall, minimal evidence for hepatic, cardiovascular, renal, and reproductive organ alterations was observed. In the liver, focal necrosis, pigmented macrophages, and minimal to mild fibrosis were observed. Chronic periportal inflammation was seen in most animals, but a slight increase in severity was suggested at HD. Inflammation of the heart and/or vessels was observed. In the kidney, chronic interstitial inflammation medullary tubule cyst, eosinophilic cast, and mild congestion were observed. In the spleen, mild serosal fibrosis was observed in treated females. Lactation of the mammary gland, ovum mineralization in the ovary, and minimal chronic inflammation of the cervix were observed. Although observed in all groups, increased severity of inflammation of the tongue and esophagus were observed in treated animals.

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6M Interim	Males				Females			
Organ/Finding	**Con (3)**	**LD (3)**	**MD (2)**	**HD (3)**	**Con (3)**	**LD (3)**	**MD (3)**	**HD (3)**
Liver								
Periportal fibrosis	0	0	0	0	0	0	2	1
Heart								
Chronic inflammation, myocardium	0	1	0	1	0	1	2	1
Aorta								
Chronic inflammation, adventia	0	0	0	0	0	0	0	1
Vacuolation muscular wall	0	0	0	0	0	0	0	1
Kidney								
Chronic interstitial inflammation	1	3	2	0	2	2	1	3
Med. tubule cyst	0	0	0	1	0	0	0	0
Eosinophilic cast	0	0	0	0	0	0	0	1
Congestion	0	0	0	0	0	0	0	1
Mammary Gland								
Lactation					0	0	0	1*
Ovary								
Ovum mineralization					1	3	2	2
Cervix								
Chronic inflammation					0	0	0	1

*This HDF was found dead at 7 months, not interim sacrifice

12M Terminal	Males				Females			
Organ/Finding	**Con (4)**	**LD (4)**	**MD (4)**	**HD (4)**	**Con (4)**	**LD (4)**	**MD (4)**	**HD (3)**
Liver								
Focal necrosis	0	0	0 (+1 at 6M)	1	0	0	0	0
Focal pigmented macrophages	0	0	0	1	0	0	0	0
Focal serosal fibrosis	0	0	0	0	0	0	0	1
Focal subserosal fibrosis	0	0	0	0	0	0	0	2
Heart								
Chronic inflammation, myocardium	1	1	1	0	0	0	0	1
Spleen								
Serosal fibrosis	0	0	0	0	0	2	2	1
Kidney								
Chronic interstitial inflammation	1	3	4	2	4	2	2	2
Medullary tubule cyst	0	0	0	1	0	0	0	0
Pineal gland								
Focal necrosis	0	0	0	1	0	0	0	0

Toxicokinetics [D1, Week 26, and Week 52]
Plasma concentrations of BMS-214778 were determined after the first dose and after a daily dose during Weeks 26 and 52 for those tasimelteon-treated animals (four/sex/group) designated for the twelve-month phase of the study. Blood samples were collected from the femoral vein (using K3 EDTA) at approximately 1, 2, 3, 5, 7, 12, and 24 hours after the first dose and after a daily dose during Weeks 26 and 52. GLP compliance for the toxicokinetic report was not provided.

Dose-related increases in exposure were observed; however, the exposures were greater than dose-proportional between LD and HD. No clear sex difference was observed at LD (except on D1) and HD, but exposures were greater in males than in females at MD. There was some reduction in the systemic exposures with repeated once-daily dosing for one year.

Dose[A]	Study Day	CMAX [ng/mL]		TMAX [h][B]		AUC(0-T) [ng•h/mL][C]	
		Male	Female	Male	Female	Male	Female
3	1	143 (76)	243 (117)	1.0 (1.0, 2.0)	1.0 (1.0, 1.0)	273 (148)	505 (264)
	182	260 (125)	217 (229)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	373 (165)	343 (346)
	363	181 (7.6)	222 (201)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	384 (96)	352 (331)
20	1	5720 (1317)	3575 (1686)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	23931 (16482)	8750 (1952)
	182	5549 (902)	3355 (1801)	1.0 (1.0, 2.0)	1.0 (1.0, 1.0)	13755 (3628)	8439 (5136)
	363	2886 (890)	2619 (1438)	2.5 (1.0, 3.0)	1.0 (1.0, 2.0)	12792 (4520)	6281 (2232)
150	1	26889 (2662)	27313 (8324)	2.5 (2.0, 5.0)	3.5 (2.0, 5.0)	243539 (63912)	287024 (117961)
	182	25270 (12887)	21144 (6366)	2.0 (1.0, 5.0)	4.5 (1.0, 7.0)	137156 (17632)	155615 (86082)
	363	17164 (9330)	20258 (4369)[D]	4.0 (2.0, 12)	3.0 (1.0, 5.0)[D]	145199 (56409)	139243 (69691)[D]

[A]Expressed as mg/kg/day; [B]Median (minimum, maximum); [C]Time (T) ranged from 2 h to 24 h; [D]N=3

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Study Day	CMAX ratio		AUC(0-T) ratio	
	Male	Female	Male	Female
1	1:40:188	1:15:112	1:88:892	1:17:568
182	1:21:97	1:15:97	1:37:368	1:25:454
363	1:16:95	1:12:91	1:33:378	1:18:396

7 Genetic Toxicology

Previously (see Dr. Atrakchi's review dated 1/16/98), it was determined that tasimelteon was neither mutagenic nor clastogenic. It is noted that the rat micronucleus assay was conducted as part of the 1-month toxicity study with tasimelteon and did not use a positive control; the assay showed no increase in micronuclei in PCE at up to 400 mg/kg. A chromosomal aberration assay was not available at the time; it is reviewed below. Tasimelteon demonstrated clastogenic effects in an *in vitro* chromosomal aberration assay in HPBL. Additionally, the sponsor has investigated the genotoxicity of metabolite M11 (which has poor coverage overall in the toxicology species and is not formed *in vivo* in rat). Metabolite M11 was not mutagenic in an Ames assay but was clastogenic in an *in vitro* chromosomal aberration assay in CHO cells.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: CYTOGENETICS STUDY IN PRIMARY HUMAN LYMPHOCYTES	
Study no.:	97680
Study report location:	EDR
Conducting laboratory and location:	BMS (Syracuse, NY)
Date of study initiation:	12/1/97
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-214778 (BMS-214778-01), Batch # C014A, purity 99.2%

Methods	
Cell line:	HPBL
Concentrations in definitive study:	5 hr (+S9): 0, 100, 200, 400, and 800 μg/mL 24 hr (-S9): 0, 25, 50, 100, and 200 μg/mL
Basis of concentration selection:	A range-finding assay was performed, testing 1 - 2400 μg/mL (the upper limit was based on drug solubility). Concentration-related mitotic index reductions were seen, 37% at 100 μg /mL and 64% at 300 μg /mL (24 hr [-S9]) and 30% at 600 μg /mL and 100% at 1200 μg /mL (5 hr [+S9]).
Negative control:	DMSO ([redacted] (b) (4) Lot #01735JQ)
Positive control:	0.1 μg /mL mitomycin C (Lot No. 25H0619, [redacted] (b) (4) 4 μg /mL cyclophosphamide (Lot No. 43H0269, [redacted] (b) (4) Both prepared in sterile water
Formulation/Vehicle:	DMSO [redacted] (b) (4)
Incubation & sampling time:	37°C for 5 or 24 hr

Study Validity

The metabolic activation system used was Aroclor 1254-induced rat liver S9 fraction, purchased commercially from [redacted] (b) (4). (Note that rats in vivo do not produce metabolite M11.) The 24 hr (-S9) exposures were conducted in tissue culture flasks. The 5 hr (+S9) exposures were conducted in 15 mL centrifuge tubes, followed by two washes, then resuspended and transferred tissue culture flasks for an additional day of culture. Colcemid was used to arrest the mitotic cells in metaphase, and the flasks were incubated for an additional 3 hr. The sponsor's criteria for a positive result were based on statistical significance.

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Results

The sponsor stated that BMS-214778 was not clastogenic when tested at the maximum cytotoxicity levels "recommended by ICH guideline"; however, the mean number of cells with aberrations and the aberrations per cell suggested an increase in the 24-hr cultures (without metabolic activation) and were more than double those of the vehicle control in the 5-hr cultures (with metabolic activation). The increase in chromosome aberrations occurred only at the highest concentration, was seen in the presence of cytotoxicity (mitotic indices of 45% in 24-hr and 24% in 5-hr cultures, compared to vehicle control), and did not reach statistical significance. The observed cytotoxicity in the 24-hr cultures (without metabolic activation) did not greatly exceed the recommended maximum of 50%). The cytotoxicity in the 5-hr cultures in the presence of metabolic activation was excessive (much greater than a 50% reduction) at 800 µg/mL, but did not show a reduction in mitotic index at the next lower dose; doses between 400 and 800 µg/mL should have been tested. While not reaching statistical significance, the results should be considered equivocal/positive (increased aberrations in the presence cytotoxicity).

Cytogenetic Data Summarized by Dose Group

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TREATMENT			ABERRATIONS							
			CELLS WITH ABERRATIONS		CHROMATID		CHROMOSOME			
	MITOTIC INDEX MEAN±S.E.M	CELLS SCORED	MEAN% ± S.E.M.	ABS/CELL[1]± S.E.M.	BKS[2]	EXC[3]	BKS[2]	EXC[3]	>10[4]	TOTAL ABS[5]
DMSO	11.8% ±0.6%	200	3.0±0.6	0.04±0.01	3	0	4	0	0	7
BMS-214778 (µg/ml) 24-HOUR EXPOSURE										
25	10.7% ±0.4%	200	4.5±1.5	0.05±0.02	7	0	2	0	0	9
50	10.3% ±0.5%*	200	3.0±1.3	0.03±0.01	5	0	1	0	0	6
100	9.3% ±0.9%*	200	3.5±2.9	0.04±0.03	4	0	3	0	0	7
200	5.3% ±0.6%**	200	6.0±1.4	0.07±0.02	12	0	1	0	0	13
MITOMYCIN C (µg/ml)										
0.10	6.9% ±0.2% **	200	44.0±2.4**	0.71±0.08	81	14	47	0	0	142
DMSO+S-9	14.1% ±0.7%	200	5.5±1.0	0.06±0.01	9	0	2	0	0	11
BMS-214778 (µg/ml) 5-HOUR EXPOSURE WITH S-9										
100	13.1% ±0.8%	200	3.5±1.0	0.04±0.01	4	0	3	0	0	7
200	12.5% ±1.2%	200	3.5±0.5	0.04±0.00	5	0	2	0	0	7
400	13.2% ±0.7%	200	3.5±1.3	0.04±0.01	2	0	5	0	0	7
800	3.4% ±0.9%**	200	9.5±1.3	0.11±0.02	17	1	4	0	0	22
CYCLOPHOSPHAMIDE (µg/ml) + S-9										
4	9.3% ±0.5%**	200	38.0±2.0**	0.80±0.13	106	29	25	0	0	160

* Denotes significantly different from appropriate control at $P < 0.05$ by students "t" test.
** Denotes significantly different from appropriate control at $P < 0.01$ by students "t" test.

1 Total aberrations divided by the total number of metaphases evaluated.
2 Total of all breaks including chromatid and isochromatid type.
3 Total of all exchanges including interchanges and intrachanges.
4 Total cells observed with more than 10 separate aberrations.
5 Total aberrations observed for the treatment (sum of the individual totals plus 10 times the >10 frequency) .

7.4 Other Genetic Toxicity Studies

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study Title: BACTERIAL REVERSE MUTATION TEST

Study no.:	UN0003
Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/2/09 experimental start
GLP compliance:	Yes (b) (4)
QA statement:	Yes, but the protocol was not provided.
Drug, lot #, and % purity:	Metabolite M11, Lot WH-83-34-28, 98.5% pure

Methods (see below, from the sponsor)

Concentrations in definitive study:	**Plate incorporation** 8, 40, 200, 1000, or 5000 μg/plate **Pre-incubation** 8, 40, 200, 1000, or 5000 μg/plate, except TA1537 at 1.6, 8, 40, 200, 1000, or 5000 μg/plate
Basis of concentration selection:	An initial range-finding experiment was conducted by the plate incorporation method using TA98 and WP2 uvrA in the presence and absence of S9 mix. Duplicate plates were used. M11 concentrations of 1.6, 8, 40, 200, 1000, or 5000 mg/plate were tested. After approximately 24 hr incubation at 37°C ± 3°C, the plates were examined for the presence of a complete bacterial lawn.
Formulation/Vehicle:	dimethylsulphoxide (DMSO)
Incubation & sampling time:	66 hr at 37°C ± 3°C
	S9 fraction (from Aroclor 1254-treated rats) from (b) (4) batch 2375

Bacterial Strains:

S. typhimurium	TA1535	his G46	*rfa*$^-$	Δuvr B$^-$	
S. typhimurium	TA1537	his C3076	*rfa*$^-$	Δuvr B$^-$	
S. typhimurium	TA98	his D3052	*rfa*$^-$	Δuvr B$^-$	pKM101
S. typhimurium	TA100	his G46	*rfa*$^-$	Δuvr B$^-$	pKM101
E. coli	WP2 *uvrA*	trpE	*rfa*$^+$	Δuvr A$^-$	

Positive Controls:

3.3.1 Without S-9 mix

Strain	Positive Control (PC)
TA1535	Sodium Azide (SA) 1 µg/plate
TA1537	9-Aminoacridine (9AA) 50 µg/plate
TA98	2-Nitrofluorene (2NF) 0.5 µg/plate
TA100	SA 1 µg/plate
WP2 *uvrA*	4-Nitroquinoline-N-oxide (4NQO) 1 µg/plate

3.3.2 With S-9 mix

Strain	Positive Control
TA1535	2-Aminoanthracene (2AA) 2 µg/plate
TA1537	2AA 4 µg/plate
TA98	2AA 2 µg/plate
TA100	2AA 4 µg/plate
WP2 *uvrA*	2AA 10 µg/plate

Results

The cytotoxicity range-finding assay suggested that the maximum concentration was adequate (see sponsor's table, below).

Strain	Nominal Dose µg/plate	0	1.6	8	40	200	1000	5000
TA98	- S-9	+	+	+	+	+	srl	srl
	+ S-9	+	+	+	+	+	+	+
WP2 uvrA	- S-9	+	+	+	+	+	+	srl
	+ S-9	+	+	+	+	+	+	+

+: growth of background bacterial lawn.
srl: slightly reduced background bacterial lawn.

There was one issue with the validity of the assay, as 2-AA was the sole indicator of the activity of the S9 mix; however, the study used a commercially prepared S9. In the plate incorporation and pre-incubation experiments, no drug-related increases in revertant numbers were reported. See the sponsor's summary tables, following. M11 was not a bacterial mutagen, under the conditions of the test.

Table 1 - Mean Number of Revertants Per Plate - Experiment 1 - Plate Incorporation

		Concentration of test article (µg/plate)						
Strain	% S-9 mix v/v	0	8	40	200	1000	5000	PC
TA1535	0	14.7	16.3	13.0 srl	10.0	19.7	19.3	746.7
TA1537	0	8.7	9.7	9.3	8.7 srl	8.0 srl	4.7 srl	241.0
TA98	0	27.7	24.3	20.7	22.0	27.0	30.0	94.7
TA100	0	107.3	105.7	102.0	103.7	98.3	90.3	802.3
WP2 *uvrA*	0	33.0	29.0	30.3	21.3	34.3	20.7	709.7
		Concentration of test article (µg/plate)						
Strain	% S-9 mix v/v	0	8	40	200	1000	5000	PC
TA1535	10	9.3	13.3	7.3	15.3	9.3	11.3	144.7
TA1537	10	11.3	8.0	8.3	11.0	11.7	9.3	121.7
TA98	10	32.0	28.3	30.0	27.7	33.0	31.3	103.3
TA100	10	115.0	94.3	95.0	83.0	89.0	25.3	505.3
WP2 *uvrA*	10	37.3	44.0	37.0	40.0	38.0	21.0	100.7

PC: positive control.
srl: slightly reduced background bacterial lawn.

Table 2 - Mean Number of Revertants Per Plate - Experiment 2 - Pre-Incubation

			Concentration of test article (µg/plate)						
Strain	% S-9 mix v/v	0	1.6	8	40	200	1000	5000	PC
TA1535	0	17.7	NT	19.0	16.0	16.7	13.3	19.0	786.3
TA1537	0	8.7	6.3	9.0	7.7	8.3	11.7	15.7	86.0
TA98	0	26.3	NT	21.7	22.7	25.3	28.0	25.0	107.0
TA100	0	103.0	NT	98.3	102.7	100.0	100.0	112.7	740.7
WP2 *uvrA*	0	25.7	NT	26.0	26.3	29.0	31.7	27.7	1422.3
			Concentration of test article (µg/plate)						
Strain	% S-9 mix v/v	0	1.6	8	40	200	1000	5000	PC
TA1535	10	12.0	NT	12.3	11.3	12.7	13.7	11.0	229.3
TA1537	10	13.3	NT	17.0	13.7	10.3	10.3	11.0	122.3
TA98	10	29.7	NT	32.3	33.0	30.3	31.3	25.7	723.7
TA100	10	133.0	NT	132.0	113.0	118.7	128.3	108.0	2324.0
WP2 *uvrA*	10	40.7	NT	40.0	35.3	38.0	42.0	31.3	283.3

PC: positive control.
NT: not tested.

Positive Control Data for Spontaneous Revertants in the Presence of S9							
Strain	Number	Mean	Standard Deviation	Mean -2s.d.	Mean +2s.d.	Minimum	Maximum
TA98 2AA (2.0 µg/plate)	108	581.9	392.9	-203.9	1367.7	85	1990
TA100 2AA (4.0 µg/plate)	105	1080.0	499.1	81.8	2078.2	234	2388
TA1535 2AA (2.0 µg/plate)	108	105.7	36.2	33.3	178.2	44	200
TA1537 2AA (4.0 µg/plate)	108	118.5	53.5	11.5	225.6	35	327
WP2 *uvrA* 2AA (10 µg/plate)	108	202.8	82.1	38.7	366.9	58	475
Positive Control Data for Spontaneous Revertants in the Absence of S9							
Strain	Number	Mean	Standard Deviation	Mean -2s.d.	Mean +2s.d.	Minimum	Maximum
TA98 2NF (0.5 µg/plate)	105	126.9	42.2	42.5	211.3	67	237
TA100 SA (1.0 µg/plate)	106	621.7	166.3	289.1	954.3	239	965
TA1535 SA (1.0 µg/plate)	107	644.0	163.9	316.1	971.9	90	968
TA1537 9AA (50.0 µg/plate)	106	242.0	147.4	-52.8	536.8	36	734
WP2 *uvrA* 4NQO (1.0 µg/plate)	108	1107.8	511.2	85.3	2130.3	310	2601

In Vitro Assays in Mammalian Cells

Study Title*: IN VITRO* MAMMALIAN CELL CYTOGENETIC TEST: CHINESE HAMSTER OVARY CELLS

Study no.: UN0004

Study report location: EDR

Conducting laboratory and location: (b) (4)

Slide scoring: (b) (4)

Date of study initiation: 4/7/09 experimental start

GLP compliance: Yes, pg 5 (b) (4)

QA statement: Yes (except slide scoring, (b) (4) QA), but the protocol was not provided.

Drug, lot #, and % purity: Metabolite M11, lot WH-83-34-28, 98.5% pure

Methods (see the sponsor's tables, below)

Cell line: CHO cells

Basis of concentration selection: Maximum concentration by solubility limit

A range-finder tested M11 concentrations of 1.6, 8, 40, 200, or 1000 mg/mL. The 5000 mg/mL concentration was not tested due to excessive precipitation.

Formulation/Vehicle: dimethylsulphoxide (DMSO)

Incubation & sampling time: Approximately 3 or 15 hr treatments
Harvested at 15-39 hr
37°C ± 3°C

S9 fraction (from Aroclor 1254-treated rats) from [redacted] (b) (4) batch 2375

Positive Controls

Metabolic Activation	Positive Control	Final Concentration(s)
10 % v/v S-9 mix	BP	4 and 5 µg/mL
10 % v/v S-9 mix	CPA	10 µg/mL
None	MMC	0.2 and 0.25 µg/mL

Treatments (3 experiments)

Experiment 1

Treatment Duration	Metabolic Activation	Serum Content
3 hours	10 % v/v S-9 mix added at 10 % (v/v)	None
3 hours	None	None
1.5 cell cycles	None	10 % v/v FBS

Experiment 2

Metabolic Activation	Serum content	Treatment time (hours)	Harvest time (hours after treatment began)	
			Harvest 1	Harvest 2
10 % v/v S-9 mix	None	3	15	39
None	10 % v/v	15	15	39

Experiment 3

Metabolic Activation	Serum content	Treatment time (hours)	Harvest time (hours after treatment began)	
			Harvest 1	Harvest 2
10 % v/v S-9 mix	None	3	15	39

Study Validity

The mitotic index (MI) for each culture was determined from a minimum of 1000 cells; the doses used for scoring were based on cytotoxicity of 50-75%. Cytotoxicity was based on colony forming capacity (CFC). One hundred cells were counted from each culture for scoring.

Results

M11 was clastogenic in the presence of metabolic activation. Although the clastogenic response was not consistent throughout experiments, it was reproducible and dose-dependent. M11 was negative in experiment 1, but was positive in experiments 2 and 3 (particularly 2nd harvest). The sponsor described the clastogenic response as "biologically relevant and reproducible" at the second harvest in the presence of S9 mix, and indicated that "M11 has the potential to delay cell cycling, which is confirmed by the potential increases in polyploidy, endoreduplication and hyperdiploidy (also observed in the absence of S-9 mix following 1.5 cell cycle exposure)". Generally, M11 was not clastogenic in the absence of metabolic activation.

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Table 6 - Experiment 1 Colony Forming Capacity

	Relative CFC %		Relative CFC %
Dose µg/mL	Presence of metabolic activation 3 hour treatment	Dose µg/mL	Absence of metabolic activation 3 hour treatment
0	100 %	0	100 %
10	88 %	50	105 %
200	73 %	1000	87 %
1000	62 %	1500	48 %
2000	3 %	1700	36 %
3000	Dose level discarded, due to lack of viable cells	1750	31 %
3690	Dose level discarded, due to lack of viable cells	1800	34 %
		1850	25%
		1900	18 %

Experiment 1 Aberration Data Absence of Metabolic Activation

Dose µg/mL	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Combined Data														
0	400	22	5.13	10	3	2	1	7	0	0	0	0	5.5	3.3
50	200	6	4.65	4	0	1	0	2	0	0	0	1	3.0	1.5
1000	200	10	4.15	6	1	0	0	3	0	0	0	1	5.0	2.0
1500	200	7	4.35	4	1	1	0	1	0	0	0	0	3.5	1.5
MMC 0.25	125	35	2.85	10	15	13	1	10	0	0	0	1	28.0	24.0
Culture A														
0	200	12	5.35	7	1	2	0	3	0	0	0	0	6.0	3.0
50	100	2	4.90	1	0	0	0	1	0	0	0	0	2.0	1.0
1000	100	4	4.20	3	0	0	0	1	0	0	0	1	4.0	1.0
1500	100	4	4.20	1	1	1	0	1	0	0	0	0	4.0	3.0
MMC 0.25	100	27	2.90	8	11	12	1	7	0	0	0	1	27.0	22.0
Culture B														
0	200	10	4.90	3	2	0	1	4	0	0	0	0	5.0	3.5
50	100	4	4.40	3	0	1	0	1	0	0	0	1	4.0	2.0
1000	100	6	4.10	3	1	0	0	2	0	0	0	0	6.0	3.0
1500	100	3	4.50	3	0	0	0	0	0	0	0	0	3.0	0.0
MMC 0.25	25	8	2.80	2	4	1	0	3	0	0	0	0	32.0	32.0

MMC = Mitomycin C

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Experiment 1 Aberration Data Presence of Metabolic Activation

Dose µg/mL	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Combined Data														
0	400	19	6.65	13	2	0	3	2	0	0	0	1	4.8	1.8
10	200	7	6.15	7	0	0	0	1	0	1	0	0	3.5	0.5
200	200	12	6.10	8	2	0	0	2	0	0	0	1	6.0	2.0
1000	200	5	5.55	1	0	0	2	3	0	1	0	0	2.5	2.0
BP 5	50	19	4.55	1	7	17	3	0	0	3	0	0	38.0	36.0
Culture A														
0	200	11	7.10	8	2	0	1	1	0	0	0	1	5.5	2.0
10	100	2	6.60	2	0	0	0	1	0	0	0	0	2.0	1.0
200	100	6	5.80	6	0	0	0	0	0	0	0	0	6.0	0.0
1000	100	1	5.40	0	0	0	1	1	0	1	0	0	1.0	1.0
BP 5	25	9	5.40	0	5	9	0	0	0	1	0	0	36.0	36.0
Culture B														
0	200	8	6.20	5	0	0	2	1	0	0	0	0	4.0	1.5
10	100	5	5.70	5	0	0	0	0	0	1	0	0	5.0	0.0
200	100	6	6.40	2	2	0	0	2	0	0	0	1	6.0	4.0
1000	100	4	5.70	1	0	0	1	2	0	0	0	0	4.0	3.0
BP 5	25	10	3.70	1	2	8	3	0	0	2	0	0	40.0	36.0

BP = Benzo(a)Pyrene

Table 8 - Experiment 2 Colony Forming Capacity

	Relative CFC %		Relative CFC %
Dose µg/mL	Presence of metabolic activation 3 hour treatment	Dose µg/mL	Absence of metabolic activation 1.5cc treatment
0	100 %	0	100 %
50	107 %	1	109 %
1000	130 %	10	134 %
1500	97 %	200	121 %
1700	58 %	1000	71 %
1750	55 %	1500	48 %
1800	50 %		
1850	41 %	2000	0 %
1900	24 %		

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Experiment 2 Aberration Data Absence of Metabolic Activation 1st Harvest

Dose µg/mL	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Combined Data														
0	400	6	4.58	1	3	1	0	1	0	10	0	0	1.5	1.3
10	200	2	4.30	0	2	0	0	0	0	6	0	0	1.0	1.0
200	200	0	3.10	0	0	0	0	0	0	5	0	0	0.0	0.0
1000	200	3	4.15	0	2	0	0	1	0	7	0	0	1.5	1.5
1500	200	2	1.45	0	0	0	1	1	0	2	0	0	1.0	1.0
MMC 0.25	50	18	2.85	2	7	16	3	0	0	0	0	0	36.0	36.0
Culture A														
0	200	3	4.50	1	2	0	0	0	0	6	0	0	1.5	1.0
10	100	0	3.80	0	0	0	0	0	0	3	0	0	0.0	0.0
200	100	0	3.00	0	0	0	0	0	0	1	0	0	0.0	0.0
1000	100	3	4.20	0	2	0	0	1	0	5	0	0	3.0	3.0
1500	100	2	1.50	0	0	0	1	1	0	2	0	0	2.0	2.0
MMC 0.25	25	10	2.40	1	2	8	2	0	0	0	0	0	40.0	40.0
Culture B														
0	200	3	4.65	0	1	1	0	1	0	4	0	0	1.5	1.5
10	100	2	4.80	0	2	0	0	0	0	3	0	0	2.0	2.0
200	100	0	3.20	0	0	0	0	0	0	4	0	0	0.0	0.0
1000	100	0	4.10	0	0	0	0	0	0	2	0	0	0.0	0.0
1500	100	0	1.40	0	0	0	0	0	0	0	0	0	0.0	0.0
MMC 0.25	25	8	3.30	1	5	8	1	0	0	0	0	0	32.0	32.0

MMC = Mitomycin C

Experiment 2 Aberration Data Absence of Metabolic Activation 2nd Harvest

Dose µg/mL	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Combined Data														
0	400	15	4.78	1	9	1	0	4	0	14	2	0	3.8	3.5
1500	200	9	3.30	1	5	5	0	0	0	14	4	0	4.5	4.0
Culture A														
0	200	8	4.60	0	4	1	0	3	0	5	0	0	4.0	4.0
1500	100	5	3.90	1	2	3	0	0	0	6	1	0	5.0	4.0
Culture B														
0	200	7	4.95	1	5	0	0	1	0	9	2	0	3.5	3.0
1500	100	4	2.70	0	3	2	0	0	0	8	3	0	4.0	4.0

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Experiment 2 Aberration Data Presence of Metabolic Activation 1st Harvest

Dose µg/mL	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Combined Data														
0	400	6	4.73	1	3	0	0	2	0	9	0	3	1.5	1.3
50	200	1	4.65	0	0	0	0	1	0	11	0	1	0.5	0.5
1500	200	7	3.80	0	4	1	1	1	0	6	0	1	3.5	3.5
1800	200	28	2.85	0	20	13	1	2	2	7	0	1	14.0	14.0
CPA 10	125	27	2.35	0	21	12	0	0	0	3	0	0	21.6	21.6
Culture A														
0	200	2	4.55	1	1	0	0	0	0	3	0	3	1.0	0.5
50	100	0	4.90	0	0	0	0	0	0	4	0	0	0.0	0.0
1500	100	2	3.50	0	2	0	0	0	0	2	0	1	2.0	2.0
1800	100	15	3.40	0	9	7	1	0	2	3	0	0	15.0	15.0
CPA 10	100	19	2.00	0	15	5	0	0	0	1	0	0	19.0	19.0
Culture B														
0	200	4	4.90	0	2	0	0	2	0	6	0	0	2.0	2.0
50	100	1	4.40	0	0	0	0	1	0	7	0	1	1.0	1.0
1500	100	5	4.10	0	2	1	1	1	0	4	0	0	5.0	5.0
1800	100	13	2.30	0	11	6	0	2	0	4	0	1	13.0	13.0
CPA 10	25	8	2.70	0	6	7	0	0	0	2	0	0	32.0	32.0

CPA = Cyclophosphamide

Experiment 2 Aberration Data Presence of Metabolic Activation 2nd Harvest

Dose µg/mL	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Combined Data														
0	400	5	5.53	0	4	0	0	1	0	4	0	0	1.3	1.3
1800	200	28	3.65	0	16	20	0	4	1	11	3	1	14.0	14.0
Culture A														
0	200	1	5.65	0	1	0	0	0	0	1	0	0	0.5	0.5
1800	100	18	4.00	0	11	13	0	1	1	6	2	1	18.0	18.0
Culture B														
0	200	4	5.40	0	3	0	0	1	0	3	0	0	2.0	2.0
1800	100	10	3.30	0	5	7	0	3	0	5	1	0	10.0	10.0

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Table 10 - Experiment 3 Colony Forming Capacity

	Relative CFC %
Dose µg/mL	Presence of metabolic activation 3 hour treatment
0	100 %
1400	63 %
1500	34 %
1600	11 %
1700	9 %
1800	5 %

Experiment 3 Aberration Data Presence of Metabolic Activation 1st Harvest

Dose µg/mL	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Combined Data														
0	400	8	3.88	2	5	0	0	1	0	11	0	6	2.0	1.5
1400	200	1	2.80	0	1	0	0	0	0	4	0	2	0.5	0.5
1500	200	8	2.90	1	3	2	1	0	1	7	0	2	4.0	3.5
CPA 10	125	26	1.60	0	12	18	1	0	0	3	0	1	20.8	20.8
Culture A														
0	200	2	3.70	0	1	0	0	1	0	4	0	2	1.0	1.0
1400	100	1	3.30	0	1	0	0	0	0	4	0	2	1.0	1.0
1500	100	1	2.80	1	0	0	0	0	0	0	0	0	1.0	0.0
CPA 10	25	8	1.60	0	3	8	1	0	0	0	0	0	32.0	32.0
Culture B														
0	200	6	4.05	2	4	0	0	0	0	7	0	4	3.0	2.0
1400	100	0	2.30	0	0	0	0	0	0	0	0	0	0.0	0.0
1500	100	7	3.00	0	3	2	1	0	1	7	0	2	7.0	7.0
CPA 10	100	18	1.60	0	9	10	0	0	0	3	0	1	18.0	18.0

CPA = Cyclophosphamide

Experiment 3 Aberration Data Presence of Metabolic Activation 2nd Harvest

Dose µg/mL	Total cells	Aberrant cells	Mitotic Index	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
					deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Combined Data														
0	400	15	4.63	10	0	0	0	5	0	0	0	0	3.8	1.3
1400	200	6	3.20	2	4	2	1	1	0	0	0	0	3.0	2.5
1500	125	20	2.90	4	8	16	1	2	1	0	0	0	16.0	13.6
Culture A														
0	200	6	4.70	4	0	0	0	2	0	0	0	0	3.0	1.0
1400	100	3	2.80	0	2	1	0	1	0	0	0	0	3.0	3.0
1500	100	12	1.90	2	2	9	0	1	0	0	0	0	12.0	10.0
Culture B														
0	200	9	4.55	6	0	0	0	3	0	0	0	0	4.5	1.5
1400	100	3	3.60	2	2	1	1	0	0	0	0	0	3.0	2.0
1500	25	8	3.90	2	6	7	1	1	1	0	0	0	32.0	28.0

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Appendix 7 - Historical Control Data (2003 to 2007)

Whilst every effort has been made to ensure the accuracy of these data, they have not been audited by the QA unit at (b) (4)

	Number of cultures analysed		Results per 100 cells scored													
			Cells analysed	Aberrant cells	Mitotic Index %	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
							deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Negative control without metabolic activation	191	mean	193.8	2.4	3.7	1.3	0.5	0.1	0.1	0.6	0.0	0.4	0.0	0.4	2.4	1.2
		SD	24.3	1.6	1.1	1.3	0.5	0.2	0.3	0.7	0.0	0.6	0.1	0.6	1.6	0.9
		mean – 2SD	145.3	-0.8	1.5	-1.3	-0.6	-0.3	-0.5	-0.7	0.0	-0.7	-0.1	-0.8	-0.8	-0.6
		mean + 2SD	242.3	5.6	5.9	3.9	1.5	0.4	0.7	1.9	0.0	1.6	0.1	1.6	5.6	2.9
		minimum	69.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		maximum	200.0	7.0	7.1	6.0	2.5	1.0	2.0	3.5	0.0	3.0	0.5	2.5	7.0	4.0
Mitomycin C	125	mean	34.7	41.5	2.1	5.1	21.4	26.2	1.6	2.6	0.5	0.3	0.0	0.4	41.5	39.1
		SD	26.2	12.2	1.1	6.4	11.9	13.0	2.9	3.6	1.8	1.0	0.0	1.3	12.2	12.1
		mean – 2SD	-17.7	17.0	0.0	-7.7	-2.4	0.1	-4.1	-4.7	-3.2	-1.7	0.0	-2.1	17.0	14.8
		mean + 2SD	87.1	66.0	4.3	17.9	45.2	52.3	7.3	9.9	4.1	2.2	0.0	3.0	66.0	63.4
		minimum	6.0	3.6	0.2	0.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0	0.0	3.6	3.6
		maximum	100.0	72.0	6.1	32.0	52.0	68.0	12.0	20.0	16.7	5.0	0.0	8.0	72.0	68.0

	Number of cultures analysed		Results per 100 cells scored													
			Cells analysed	Aberrant cells	Mitotic Index %	Gaps	Chromatid		Chromosome		Multiple aberrations	Numerical aberrations			% cells with aberrations	
							deletion	exchange	deletion	exchange		Poly	Endo	Hyper	with gaps	without gaps
Negative control with metabolic activation	179	mean	194.2	2.6	3.7	1.4	0.5	0.1	0.1	0.6	0.0	0.5	0.0	0.5	2.6	1.3
		SD	23.2	1.7	1.1	1.6	0.6	0.2	0.3	0.6	0.0	0.7	0.1	0.7	1.7	0.9
		mean – 2SD	147.8	-0.8	1.4	-1.7	-0.8	-0.4	-0.5	-0.6	0.0	-0.8	-0.2	-0.9	-0.8	-0.5
		mean + 2SD	240.6	6.0	6.0	4.5	1.8	0.5	0.7	1.8	0.0	1.8	0.2	1.8	6.0	3.1
		minimum	100.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		maximum	201.0	10.0	7.8	8.5	3.0	1.0	1.5	2.5	0.0	3.0	0.5	3.0	10.0	4.5
Benzo(a)pyrene	70	mean	51.9	33.5	2.0	2.8	11.0	27.6	1.1	3.4	0.6	0.6	0.0	0.4	33.5	31.5
		SD	36.3	14.8	1.1	4.4	9.0	18.9	2.7	3.8	3.6	1.4	0.0	1.1	14.8	15.0
		mean – 2SD	-20.7	3.8	-0.3	-5.9	-7.1	-10.1	-4.4	-4.2	-6.6	-2.2	0.0	-1.7	3.8	1.5
		mean + 2SD	124.6	63.2	4.2	11.5	29.0	65.3	6.5	11.0	7.9	3.4	0.0	2.5	63.2	61.6
		minimum	11.0	6.0	0.1	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	3.0
		maximum	100.0	76.0	5.0	24.0	52.0	96.0	20.0	12.0	29.4	7.3	0.0	4.0	76.0	68.0
Cyclophosphamide	47	mean	35.7	39.0	1.9	5.7	19.2	22.5	3.3	2.2	0.3	0.5	0.0	0.1	39.0	35.9
		SD	27.1	13.7	1.0	6.1	11.1	11.9	4.0	3.4	1.1	1.8	0.0	0.4	13.7	13.5
		mean – 2SD	-18.5	11.6	-0.2	-6.4	-3.0	-1.3	-4.8	-4.7	-1.9	-3.0	0.0	-0.8	11.6	8.9
		mean + 2SD	90.0	66.3	4.0	17.8	41.5	46.3	11.4	9.0	2.4	4.0	0.0	1.0	66.3	62.8
		minimum	16.0	7.0	0.2	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0	4.0
		maximum	100.0	64.0	4.4	24.0	48.0	44.0	15.0	14.3	5.0	10.5	0.0	2.0	64.0	62.5

8 Carcinogenicity

Study title: VEC-162: CARCINOGENICITY STUDY BY ORAL GAVAGE ADMINISTRATION TO CD RATS FOR 104 WEEKS	
Study no.:	TAJ0001
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/9/06
GLP compliance:	Yes (b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	VEC-162, batches 800156800, 800156790, 800167150, and 800167140, purity 99.1- 99.7%
CAC concurrence:	Yes (see ExecCAC minutes dated 8/23/99). Recommended doses were 20, 100, and 250 mg/kg.

Key Study Findings

FDA Statistically Significant Tumors: Uterus (F, 250 mg/kg): endometrial AC

Sponsor's Drug-related and Significant Tumors:
Liver (M & F, ≥ 100 mg/kg): adenoma (+ carcinoma in M)
Uterus (F, 250 mg/kg): endometrial AC and SCC
Uterine Cervix (F, 250 mg/kg): SCC

Adequacy of Carcinogenicity Study

Overall, the study was adequate. The study used the doses recommended by the ExecCAC (see minutes dated 8/23/99). Mortality was increased in HDF at week 102; therefore, dosing was suspended in this group. All male groups were terminated at week 103 because of low survival in the control group.

Appropriateness of Test Models

The rat is a standard species used for 2-year carcinogenicity bioassays.

Evaluation of Tumor Findings

The only tumor reaching statistical significance was the uterine endometrial adenocarcinoma in HDF (see FDA Biostatistics review by S. Thomson). However, the sponsor reported drug-related tumors in liver (hepatocellular adenoma [and carcinoma in M], ≥ MD, M and F), uterus (endometrial adenocarcinoma & squamous cell carcinoma, HD, and uterine cervix (squamous cell carcinoma, HD). According to the sponsor's statistical analysis, these tumors were statistically significant for trend, and for pairwise comparison at HD (liver adenoma in M, uterine endometrial adenocarcinoma).

Reference ID: 3399188

Methods	
Frequency of dosing:	QD
Dose volume:	2.5 mL/kg
Route of administration:	PO, gavage
Formulation/Vehicle:	100% PEG-400
Basis of dose selection:	MTD. At 100 and 400 mg/kg in a 1-month toxicity study, hyaline droplet nephropathy was present that did not resolve in males following a 1-month recovery period. In a 6-month toxicity study, one female given 500 mg/kg was sacrificed in moribund condition after 3 doses due to severe clinical signs, including seizures and labored respiration. The incidence and severity of progressive nephropathy were increased at 500 mg/kg. Therefore, 250 mg/kg was selected as the HD, with the LD and MD selected at appropriate intervals.
Species/Strain:	Crl:CD® (SD)IGS BR rats (b) (4)
Age:	22 to 28 days of age Weights: M: 70-100 g, F: 60-90 g
Animal housing:	5/cage
Paradigm for dietary restriction:	n/a, ad libitum food and water
Interim sacrifice:	All males were sacrificed at week 103 (treated for 102 weeks) based on mortality in the control group.
Deviation from study protocol:	The sponsor identified a total of 10 protocol deviations; the sponsor indicated that none of them were considered to have affected the integrity of the study.

Group	Treatment	Dose# (mg/kg/day)	No. of animals		Main study Animal numbers		Cage numbers	
			Male	Female	Male	Female	Male	Female
1	Control	0	65	65	1-65	301-365	1-13	61-73
2	VEC-162	20	65	65	76-140	376-440	16-28	76-88
3	VEC-162	100	65	65	151-215	451-515	31-43	91-103
4	VEC-162	250	65	65	226-290	526-590	46-58	106-118

Group	Treatment	Dose# (mg/kg/day)	No. of animals		Satellite study$ Animal numbers		Cage numbers	
			Male	Female	Male	Female	Male	Female
1	Control	0	10	10	66-75	366-375	14-15	74-75
2	VEC-162	20	10	10	141-150	441-450	29-30	89-90
3	VEC-162	100	10	10	216-225	516-525	44-45	104-105
4	VEC-162	250	10	10	291-300	591-600	59-60	119-120

Expressed in terms of test material as supplied.
$ Satellite animals used for Toxicokinetic and liver sampling only (culled after sampling in Week 26).

Observations and Results

Mortality [2x/daily]

There was no statistically significant treatment-related effect on mortality, although survival was slightly lower in treated (particularly HD) females. Dosing was suspended for HDF during Week 101/102 because of low survival numbers; all female groups were terminated at 104 weeks. Survival in the control male group was reduced during Week 102, and all male groups were terminated at Week 102/103. The summary data are presented below (from the sponsor). There was an increased incidence of renal disease as cause of death (COD) in VEC-162-treated males. In HDF, there were increased incidences of "poor clinical condition," endometrial carcinoma, and uterine squamous cell carcinoma as COD (deaths between Weeks 79 and 95; 4 uterine adenocarcinoma, 2 uterine SCC, 1 uterine cervix SCC).

Group distribution of mortality

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	20	100	250	0	20	100	250
Premature deaths	45	46	33	38	43	45	44	48
Percentage survival	31	29	49	42	34	31	32	26

FIGURE 1

Percentage survival versus period of treatment

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250

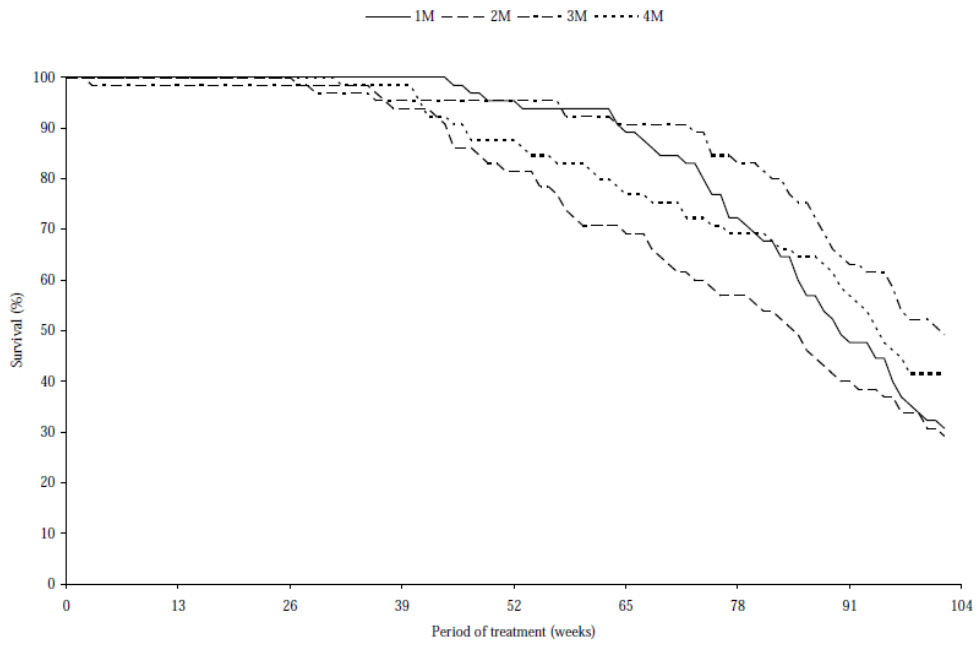
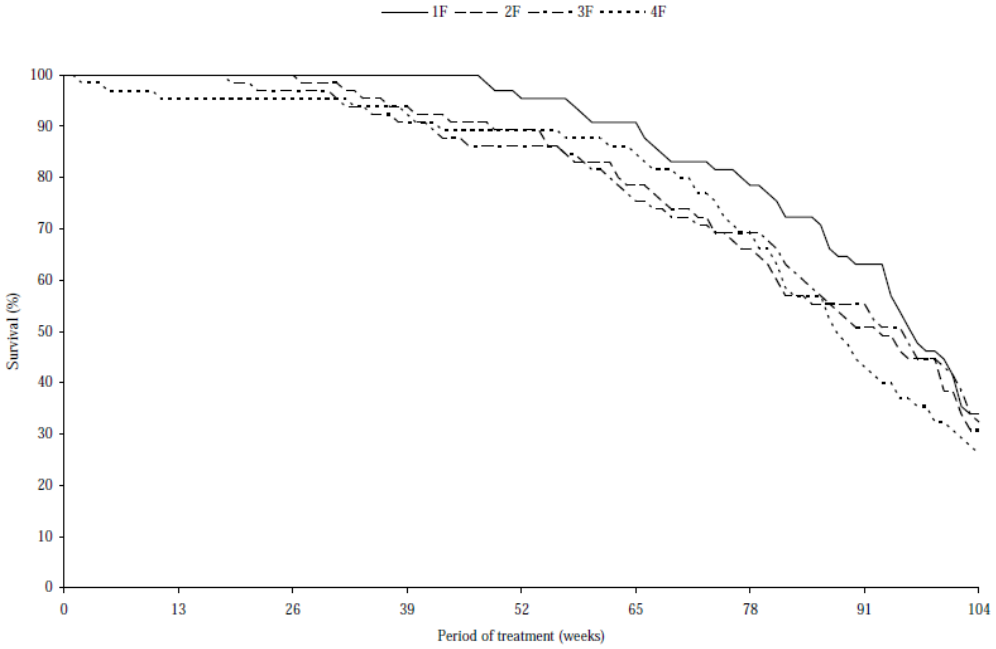


FIGURE 1 - continued

Percentage survival versus period of treatment

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250



Clinical Signs [2x/daily, with detailed physical exams weekly, and detailed observations. In addition, detailed observations were recorded daily during Week 1, twice weekly during Weeks 2-4, once weekly during Weeks 5-13, biweekly during Weeks 14-52, and once every 4 weeks thereafter at the following times: immediately before dosing, immediately after dosing, on completion of dosing of each group, between one and two hours after completion of dosing of all groups, and as late as possible in the working day.]

The sponsor reported "underactivity" in MDF, HDF, and HDM, beginning in Week 10/11 at approximately 30 minutes post-dose. This sign was occasionally reported at LD and in MDM. Chin/snout rubbing and increased salivation were also increased in tasimelteon-treated animals. Irritable, aggressive, vocalization, rales, irregular breathing, eyelid(s) partially closed, post salivation staining, hair loss, and piloerection were also occasionally reported. Observations of swollen, reddening and/or black spot(s) on tail were slightly increased in HDM. Few signs were recorded as “associated with dosing”, which is unusual for a study of such long duration.

Although not reported by the sponsor, convulsions were observed in a few animals (1 ConM, 3 LDM, 2 MDM, 1 LDF, and 3 HDF); the number of occurrences was not dose-related in males (1, 9, 2, and 0 in ConM, LDM, MDM, and HDM) but was increased in HDF (0, 1, 0, and 6 in ConF, LDF, MDF, and HDF). The severity of the sign was notable in some animals (leading to death/euthanasia); some of the events were noted

to be of prolonged occurrence or frequency. Notably, there was some internal inconsistency in how this sign was managed by the laboratory. For example, MDM #199, LDF #386, and HDF #577 were sacrificed due to "prolonged/frequent convulsions," with comments indicating the animals experienced convulsions lasting 30 to 35 seconds, while LDM #104 and HDF #559 experienced multiple convulsions of durations ranging from 30 seconds to 3 minutes. The reason for the discrepancy is uncertain.

The sponsor reported that the group distribution, multiplicity, and mean onset time of palpable swellings were unaffected by treatment (see sponsor's table below).

TABLE 3

Palpable swellings - group distribution, multiplicity and mean time of onset+

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250

Group /Sex	Multiplicity∅ 0	1	2	3	4 or more	Number of animals with swellings	Total number of swellings	Mean time of onset*
1M	36	15	10	3	1	29	49	63
2M	43	16	5	1	0	22	29	68
3M	38	13	11	2	1	27	45	71
4M	42	12	8	3	0	23	37	68
1F	26	19	10	5	5	39	87	82
2F	27	20	7	7	4	38	75	77
3F	35	13	9	2	6	30	65	76
4F	30	19	8	6	2	35	63	78

\+ Including swellings which regressed or were not positively identified at *post mortem* examination
∅ Expressed as number of animals bearing the indicated number of swellings
* In weeks to onset of first recorded swelling including those found at necropsy examination

Body Weights [Pre-dose, D1, weekly for 12 weeks, and then every four weeks]

Generally, the body weight gains of treated males and HDF were slightly lower than those of their respective controls (see the sponsor's summary data table, below). Overall, terminal body weights did not reflect a large difference. Average body weights at Week 102 were 96%, 96%, and 93% those of control males in the LD, MD, and HD groups, respectively. In females, average body weights at Week 104 were 108%, 97%, and 95% those of controls in the LD, MD, and HD groups, respectively. However, during Weeks 76 through 88, the average body weight in HDF fell to approximately 87% that of control females. For group mean body weights over the study, see sponsor's Figure 2 below.

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Group mean bodyweight gain (g)

Group and sex Dosage (mg/kg/day)	1M 0	2M 20	3M 100	4M 250	1F 0	2F 20	3F 100	4F 250
Week 0-28	468	440*	439*	456*	204	205	199	188*
As a % of Group 1	-	94	94	97	-	100	98	92
Week 28-80	150	154	141	102**	141	138	130	92**
As a % of Group 1	-	103	94	68	-	98	92	65
Week 0-102/104	572	545	545	513*	329	354	309	297
As a % of Group 1	-	95	95	90	-	108	94	90

Significant when compared to Group 1: * $p<0.05$; ** $p<0.01$

FIGURE 2

Bodyweight - group mean values versus period of treatment - males

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250

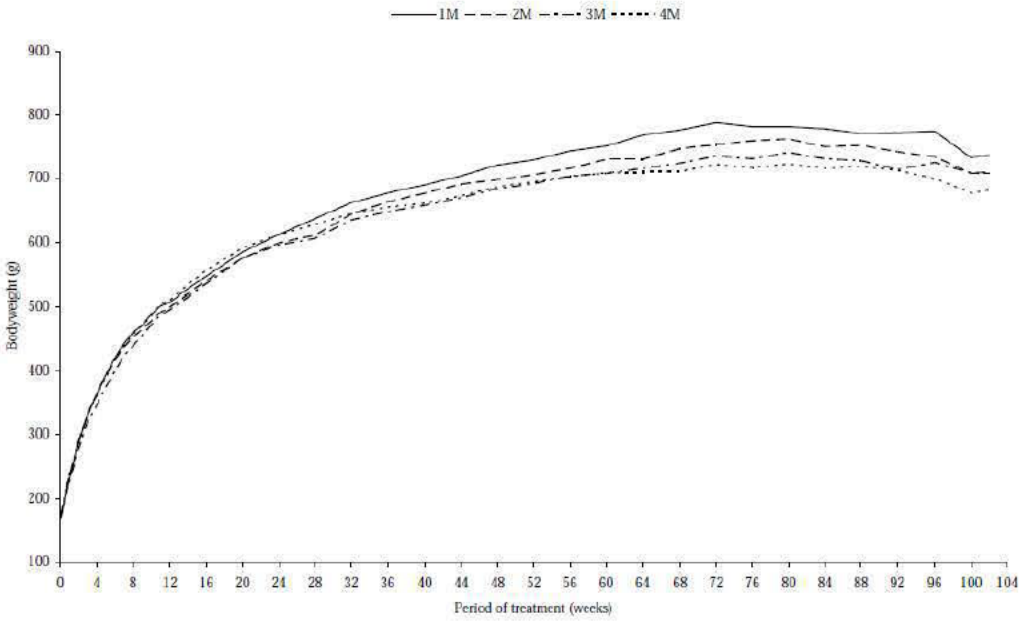
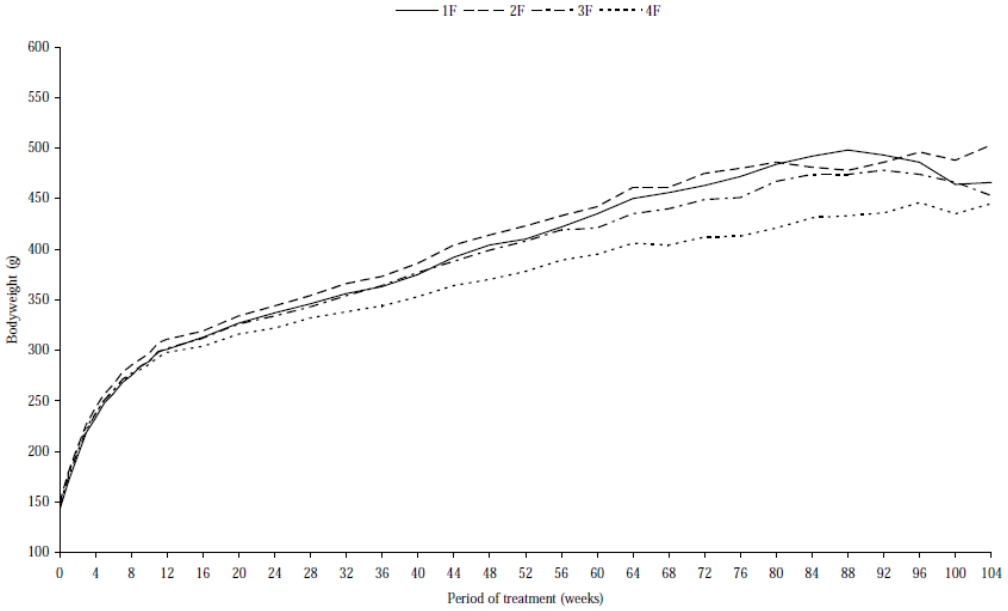


FIGURE 2 - continued

Bodyweight - group mean values versus period of treatment - females

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250



Food Consumption [Weekly for 12 weeks, and then every four weeks]

The sponsor reported that food consumption was unaffected by treatment. Food consumption values over 100 weeks of treatment were 101-103% and 105-106% of their controls in treated males and females, respectively. Generally, food consumption appeared slightly increased in treated females towards the end of the dosing period (~15%; approximately Week 92 onward).

Ophthalmology [Weeks 52 and 100]

The eyes of 20 animals/sex from the control and HD groups were examined by binocular indirect ophthalmoscope (b) (4)

No drug-related effects were reported.

Clinical Pathology [Week 52 (hematology only), Week 100 (M) or Week 101 (F)]

Blood samples were taken from all animals. Limited hematological parameters were measured, including: RBC count, wbc count with differential, PT, and APTT. Clinical chemistry were evaluated (20/sex/gp), including: ALP, ALT, AST, Bili, Urea, Creat, Gluc, Chol, Trig, Na, K, CL, Ca, Phos, Total Prot, Alb, globulins (a1, a2, Beta, Gamma), and A/G ratio. The sponsor's summary data tables for animals dying during the treatment period state that the results are "… for information only, samples analyzed outside storage stability limits."

The sponsor reported slight reductions (≤ 5%) in RBC parameters in HD animals (low RBC count only in F) during Week 52, but not at Week 100/101. MCHC was very slightly reduced in treated males at Week 52, and was reduced (~3%) in HDF at Week 101. In MD and HD females in Week 52, neutrophil counts and PT were increased very slightly (~2%). At Week 100, platelets were increased slightly (~20%) in HDM.

At Week 100/101, increased total cholesterol (as much as 35-50%) was reported at MD and HD, and was associated with increased triglycerides (as much as 65-80%) in MDM, HDM, and HDF. Alkaline phosphatase and AST were dose-dependently reduced (as much as 33%) in treated males; MD and HD females showed only a 10-25% reductions. Total protein was increased slightly (4-7%) at MD and HD; a1-globulin (13-14%, MDF, HDF, HDM) and beta-globulin (10%; MDM and HDM) showed slight increases. Changes in electrolyte levels were reported at MD and/or HD; reduced sodium (1%) and phosphorus (as much as 12%) were reported for MD and HD males, and reduced sodium and chloride (~1-2%) and increased phosphorus (~12%) were reported for HDF. Creatinine and urea were reduced 10-20% in HDF.

Urinalysis [Week 100 (M) or Week 101 (F)]

Urine samples were collected from 20 animals/sex/gp (16:00 hr until 08:30 hr the next day). Appearance, volume, pH, specific gravity, protein, U-Na, U-K, U-Cl, Gluc, Ket, Bili, UBld, and microscopy were recorded.

The sponsor reported a few urinalysis changes in MD and/or HD males. A dose-related increase in urine protein (~50-75%) was observed in tasimelteon-treated males; specific gravity was slightly increased (~2%) in HDM. Generally dose-related increase in urine sodium (~30-90%) and chloride (~50-90%) were observed in tasimelteon-treated males; increased urine sodium was also observed in HDF (~25%). Although not noted by the sponsor, average urine volume was slightly increased in females (~20%); urine protein was reduced ~20% in HDF. The incidence of trace amounts of urine ketones was increased at HD (M > F).

Organ Weights

Adjusted liver weights were increased in tasimelteon-treated males (as much as 30%), MDF, and HDF (as much as 16%). Adjusted kidney weights showed increases up to 13-14% in treated animals (dose-related in males). Adrenal weights were increased in tasimelteon-treated males, although the sponsor indicated that these increases were driven by individuals in each group. Thymus weights were slightly increased in MDM and HDM.

Gross Pathology

Macroscopic findings were reported in the liver and uterus. Enlarged liver, liver masses, and pale areas on the liver (M) were observed at MD and HD. Uterine masses were reported in HDF. See the sponsor's summary table, below.

Reference ID: 3399188

Group distribution of macroscopic findings related to treatment

Group/sex Dose (mg/kg/day)		1M 0	2M 20	3M 100	4M 250	1F 0	2F 20	3F 100	4F 250
Liver									
Enlargement	U	2	4	8	12	1	1	3	9
	T	0	0	1	3	1	0	2	0
	A	2	4	9	15	2	1	5	9
Masses	U	4	2	4	3	0	1	4	8
	T	0	0	4	5	5	3	3	3
	A	4	2	8	8	5	4	7	11
Pale areas	U	9	8	11	13	10	8	5	11
	T	6	4	11	13	4	8	5	5
	A	15	12	22	26	14	16	10	16
Uterus									
Masses	U	-	-	-	-	5	3	2	10
	T	-	-	-	-	4	4	2	7
	A	-	-	-	-	9	7	4	17

U - Unscheduled; T - Terminal kill; A - All animals

In addition to the findings reported by the sponsor, there were findings of low or slightly increased incidence of uncertain toxicological importance in several tissues, including adrenal, brain, eye, heart, kidney, liver, lung, spleen, mammary gland, pituitary, thyroid/parathyroid, reproductive organs and associated glands; these are listed in the reviewer's table, below. A few general findings, such as hair loss, thinness, and others, also appeared increased. A number of lymph nodes showed low but increased incidences of changes such as enlarged, congested, and/or cystic in tasimelteon-treated animals.

Organ/ Finding	ConM	LDM	MDM	HDM	ConF	LDF	MDF	HDF
Adrenal								
Enlarged	0	1	2	3	6	10	11	12
Brain								
Depression from Pituitary mass	11	12	19	14	46	49	47	41
Dark areas	0	0	0	1	1	0	1	3
Eyes								
Opaque	0	0	1	2	0	3	2	0
Heart								
Enlarged	1	2	2	5	0	1	1	1
Thin ventricle walls	0	0	0	2	0	0	1	0

Organ/ Finding	ConM	LDM	MDM	HDM	ConF	LDF	MDF	HDF
Kidneys								
Cysts	3	7	9	5	2	2	5	1
Enlarged	2	10	5	8	1	2	4	0
Granular	2	8	4	10	4	4	2	5
Pale	3	8	2	5	3	4	3	5
Pale areas	8	3	16	10	8	13	10	9
Dark	1	1	3	1	5	1	2	9
Pelvic Dilatation	2	4	1	7	1	2	2	3
Liver								
Cysts	2	2	5	3	3	3	2	4
Dark	0	1	0	4	0	0	4	16
Lobes Enlarged	1	2	1	3	0	0	2	2
Firm	1	0	0	1	1	1	2	2
Lobular pattern accentuated	3	3	0	1	2	1	6	5
Median cleft pale areas	14	7	10	6	8	9	9	11
Lung								
Congested	5	5	6	6	2	3	4	8
Dark	0	1	1	2	1	1	1	0
Dark areas	2	1	2	4	0	4	5	1
Spleen								
Enlarged	10	5	2	4	5	2	5	7
Swollen	4	2	1	2	1	1	3	4
Mammary								
Cysts	1	4	7	2	27	18	20	19
Pituitary								
Cysts	0	2	2	5	0	1	1	0
Mass	16	20	28	19	55	55	55	48
Parathyroids								
Enlarged	0	0	1	2	0	0	0	0
Thyroids								
Cysts	1	0	3	3	0	2	2	2
Masses	1	3	1	3	0	0	0	0
Preputial glands								
Mass	0	0	0	1				
Dark areas	2	7	2	3				
Prostate								
Mass	1	1	2	3				

Reference ID: 3399188

Organ/ Finding	ConM	LDM	MDM	HDM	ConF	LDF	MDF	HDF
Seminal Vesicles								
Oedematous	0	1	0	3				
Testes								
Masses	2	1	3	6				
Clitoral Glands								
Masses					3	13	13	9
Uterus								
Abnormal Contents					3	0	1	6
Fluid distension					2	3	3	5
Ovaries								
Cystic					3	2	2	5
Masses					0	0	1	3
Periov. sac distension					1	2	4	5
Vagina								
Abnormal contents					3	2	4	5
Stomach								
Corpus thickened	7	14	9	7	4	9	4	8
Thick Limiting Ridge	4	3	2	4	0	4	6	1
Antrum white nodules	3	4	2	6	5	3	2	0
Skin, Hair loss	2	1	0	0	1	1	1	7
General, (moderate) hair loss	5	2	14	4	5	17	16	16
General, animal thin	0	0	0	0	13	8	12	18
Thorax								
Contained fluid	0	0	0	0	1	2	2	4
Abdomen								
Masses	3	1	1	1	0	1	2	3

Histopathology

Adequate Battery	**Yes (see Appendix 1)**
Separate Signed pathology report	**Yes (but limited version)**
Peer Review	**Yes, from (b) (4) and a consultant, not identified**

Neoplastic
The sponsor reported drug-related neoplasms in the liver (M & F), uterus, and uterine cervix. Increased neoplasm incidences were also suggested in ovary and mammary gland in HDF. In males, increased incidences of neoplasms were also observed in pituitary, testes, and skin.

Liver
The incidence of hepatocellular adenomas was increased at MD and HD in both sexes (9.2 and 12.3% for males and 9.2 and 10.8% for females respectively; carcinomas were also seen in MDM (but this incidence, 4%, was within the relevant historical control range (study range: 0-5% in males [mean= 1.88%, N=744])). The incidence of hepatocellular adenomas was above the historical background range from contemporaneous studies at these laboratories (study range: 0-5% in males [mean= 2.15%, N=744] and 0-4.6% in females [mean= 1.34%, N=744]). See the sponsor's summary table, below. Also see the biostatistical evaluation by FDA reviewer S. Thomson (Table 3 excerpt, below). The sponsor considered the development of these hepatic tumors to be either a secondary response to microsomal enzyme induction (which would not be relevant to humans) or "increased hepatocellular turnover."

Summary of treatment related findings in the liver for all rats

Group/sex Dose (mg /kg/day)	1M 0	2M 20	3M 100	4M 250	1F 0	2F 20	3F 100	4F 250
Hepatocellular carcinoma	0	0	2	0	0	0	0	0
Hepatocellular adenoma	2	2	6	8†#	2	1	6#	7#
Hepatocellular adenoma and carcinoma combined	2	2	8#	8†#	2	1	6	7
Number of animals examined	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**

† One-tailed pairwise comparisons against control.
# One-tailed trend test using groups up to the respective row.

Table 3. Potentially Statistically Significant Neoplasms in Rats

Sex/ organ/ tumor	Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Rats								
LIVER								
# Evaluated	65	65	65	65				
Adj. # at Risk	43.7	36.1	49.9	43.2				
HEPATOCELLULAR ADENOMA	2	2	6	8	.0123	.0444	.1802	.6222
Adj. # at Risk	43.7	36.1	50.7	43.2				
Hepatocellular Adenoma/Carcinoma	2	2	8	8	.0145	.0444	.0746	.6222
Female Rats								
LIVER								
# Evaluated	65	65	65	65				
Adj. # at Risk	45.4	39.6	40.5	40.4				
HEPATOCELLULAR ADENOMA	2	1	6	7	.0087	.0539	.0980	.8511

Female (and Male) Reproductive Organs

Neoplasms and/or other proliferative effects were seen in uterus and uterine cervix, and possibly ovary and mammary gland in females. The sponsor stated that similar effects were not observed in mice, and posited the effects were due to a “species-specific effect of unknown etiology on aging female rats.” The sponsor attributed the uterine tumors to a rat-specific effect on prolactin, which they suggested would lack human relevance. There was limited evidence of hyperplasia and neoplastic effects in male reproductive organs (i.e., see hyperplasia and interstitial cell adenoma in testes, following).

Uterus

The incidences of endometrial adenocarcinoma and uterine squamous cell carcinoma were increased in HDF; these tumors were identified as drug-related causes of death by the pathologist. See the sponsor’s summary table, below. The sponsor stated that the incidences of endometrial adenocarcinoma and squamous cell carcinoma (17.2 and 4.7%, respectively) were above the relevant historical control range (study range for adenocarcinoma 0-3.1% [mean= 0.67%] and for squamous cell carcinoma 0-0% [N=744]). Also see the statistical evaluation by FDA biostatistical reviewer S. Thomson (Table 3 excerpt, below). The sponsor indicated that uterine tumors are common in rats treated with dopamine agonists, which reduce prolactin levels; this mechanism is not believed to be relevant to humans. The sponsor further argued that chronic activation of melatonin receptors in the pars tuberalis (cf. Morgan and Williams, 1996) may similarly lower prolactin levels in rats; however, the sponsor provided no data (i.e., serum prolactin levels) to support this assertion.

Reference ID: 3399188

Summary of treatment related findings in the uterus for all rats

Group/sex Dose (mg /kg/day)	1F 0	2F 20	3F 100	4F 250
Endometrial adenoma	1	0	0	0
Endometrial adenocarcinoma	2	2	2	11†#
Endometrial adenoma and endometrial adenocarcinoma combined	3	2	2	11†#
Squamous cell carcinoma	1	0	1	3#
Animals with malignant uterine tumours	3	2	3	15
Number of animals examined	**64**	**65**	**64**	**64**

† One-tailed pairwise comparisons against control.
# One-tailed trend test using groups up to the respective row.

Table 3. Potentially Statistically Significant Neoplasms in Rats

Sex/ organ/ tumor	Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
UTERUS								
# Evaluated	64	65	64	64				
Adj. # at Risk	45.3	39.7	39.3	40.6				
ENDOMETRIAL ADENOCARCINOMA	2	2	2	11	.0002	.0035	.6360	.6360
Adj. # at Risk	45.3	39.7	39.3	40.6				
Endo. Adenoma/-Adenocarcinoma	3	2	2	11	.0005	.0102	.7722	.7722
[Adj. # at Risk	*45.5*	*39.6*	*39.5*	*39.9*				
SQUAMOUS CELL CARCINOMA	*1*	*0*	*1*	*3*	*.0543*	*.2558*	*.7160*	*1]*

Uterine Cervix

Two HDF showed squamous cell carcinoma of the uterine cervix. The incidence (3.1%) was above the historical background range from contemporaneous studies (range 0-0% [N=744]). There was a statistically significant positive trend. See excerpt from biostatistical review by S. Thomson (below).

Summary of treatment related findings in the uterine cervix for all rats

Group/sex Dose (mg /kg/day)	1F 0	2F 20	3F 100	4F 250
Squamous cell carcinoma	0	0	0	2#
Number of animals examined	**64**	**65**	**64**	**64**

One-tailed trend test using groups up to the respective row.

Table 3. Potentially Statistically Significant Neoplasms in Rats

Sex/ organ/ tumor	Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
UTERINE CERVIX								
# Evaluated	64	65	64	64				
Adj. # at Risk	45.2	39.6	39.3	39.3				
SQUAMOUS CELL CARCINOMA	0	0	0	2	.0568	.2126	.	.

Additionally, the observed squamous cell carcinomas in uterus and in uterine cervix should be combined for evaluation, yielding an incidence of 1, 0, 1, and 5; this incidence was considered positive considering that the tumors would be considered rare (the sponsor's provided historical control incidences are zero in both tissues). See excerpt from the biostatistical review by S. Thomson, below.

Table 3. Potentially Statistically Significant Neoplasms in Rats

Sex/ organ/ tumor	Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Uterus(w/cervix)								
# Evaluated	64	65	64	64				
Adj. # at Risk	45.5	39.6	39.5	40.8				
SQUAMOUS CELL CARCINOMA	1	0	1	5	.0059	.0765	.7160	1

Ovaries

Two HDF females showed a benign sertoliform tubular adenoma. The sponsor indicated that the incidence (3.0%) was within the background range for this tumor (study range 0-8.3% [mean= 1.08%, N=744]). The combination of ovarian stromal tumors was assessed. See the FDA statistical reviewer's assessment below (Table 3 excerpt). Although the trend test was close to reaching statistical significance, no pairwise comparison was found significant.

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250

Tissue and Finding	Group/Sex: Number:	1F 65	2F 65	3F 65	4F 65
Ovaries	Number Examined:	64	65	64	63
B-Sertoliform Tubular Adenoma		0	0	0	2
B-Thecal Cell tumour		0	0	1	0

Table 3. Potentially Statistically Significant Neoplasms in Rats

Sex/ organ/ tumor	Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
OVARIES								
# Evaluated	64	65	64	63				
Adj. # at Risk	45.2	39.6	39.3	38.2				
SERTOLIFORM TUBULAR ADENOMA	0	0	0	2	.0546	.2066	.	.
Adj. # at Risk	45.2	39.6	39.4	38.2				
Thecal Cell Tmr/Serto.Tub.Adenoma	0	0	1	2	.0525	.2066	.4643	.

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Mammary Gland

Although considered an incidental finding by the sponsor, the incidence of mammary adenocarcinoma was increased in HDF (and there was a slightly increased incidence of mammary adenoma in HDF). The sponsor noted that the HDF incidence (27.7%) was, however, within the historical background range from contemporaneous studies (study range 9.4-38.3% [mean 20.6%, N=742]). See the FDA statistical reviewer's assessment below (Table 3 excerpt). The sponsor also stated that there were no treatment-related increases in associated hyperplasia or other non-neoplastic findings to suggest a proliferative response in the mammary gland.

Summary of treatment related findings in the mammary for all rats

Group/sex Dose (mg /kg/day)	1M 0	2M 20	3M 100	4M 250	1F 0	2F 20	3F 100	4F 250
Mammary adenoma	0	0	0	0	2	2	2	4
Mammary fibroadenoma	3	1	0	0	25	30	17	22
Mammary adenocarcinoma	1	0	0	0	10	10	12	18†#
Animals with mammary tumours	4	1	0	0	33	35	26	33
Number of animals examined	**65**	**65**	**65**	**65**	**64**	**65**	**64**	**65**

† One-tailed pairwise comparisons against control.
# One-tailed trend test using groups up to the respective row.

Table 3. Potentially Statistically Significant Neoplasms in Rats

Sex/ organ/ tumor	Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Female Rats								
MAMMARY								
# Evaluated	64	65	64	65				
Adj. # at Risk	47.6	42.4	43.5	44.8				
MAMMARY ADENOCARCINOMA	10	10	12	18	.0146	.0356	.3134	.4864

Of uncertain toxicological importance, a few neoplasias were observed at low incidence and/or with unclear dose-relatedness for a few tissues in males (see excerpts from the sponsor's summary table, below). Where appropriate, hyperplastic changes in the same tissue were also noted. The FDA statistical reviewer's evaluation is excerpted from Table 3, below.

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250

Tissue and Finding	Group/Sex: Number:	1M 65	2M 65	3M 65	4M 65
Pituitary	Number Examined:	63	65	65	64
B-Adenoma, Pars Distalis		19	28	30	23
Hyperplasia - Pars Distalis, Focal		2	0	1	8
Testes	Number Examined:	65	65	65	65
B-Interstitial (Leydig) Cell Adenoma		3	1	4	6
Interstitial Cell Hyperplasia		4	4	8	8
Skin	Number Examined:	65	65	65	65
B-Fibroma		1	0	5	3

Table 3. Potentially Statistically Significant Neoplasms in Rats

Sex/ organ/ tumor	Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Rats								
PITUITARY								
# Evaluated	63	65	65	64				
Adj. # at Risk	47.6	45.6	55.0	46.9				
ADENOMA, PARS DISTALIS	19	28	30	23	.4822	.2361	.1104	.0296
[TESTES								
# Evaluated	*65*	*65*	*65*	*65*				
Adj. # at Risk	*43.3*	*35.7*	*49.2*	*43.2*				
INTERSTITIAL (LEYDIG) CELL ADENOMA	*3*	*1*	*4*	*6*	*.0589*	*.2417*	*.5735*	*.9135]*
SKIN								
# Evaluated	65	65	65	65				
[Adj. # at Risk	*43.1*	*35.7*	*50.3*	*43.4*				
FIBROMA	*1*	*0*	*5*	*3*	*.0920*	*.3080*	*.1404*	*1]*
Adj. # at Risk	43.1	35.7	50.3	43.9				
Fibroma/Sarcoma NOS/Fibrosarcoma	1	0	5	4	.0407	.1800	.1404	1

(Note: The FDA statistical reviewer did not include testes and skin fibroma in his assessment of potentially statistically significant neoplasms; these data were taken from Table A.2.1 in the body of his review to parallel this reviewer's discussion.)

Non-neoplastic

The sponsor reported non-neoplastic histopathology in the liver (both sexes), kidney (F), ovary, and uterine cervix; other organs showing non-neoplastic alterations include kidney (M), spleen, uterus, mammary, testes, and pituitary. A few other low incidence alterations or alterations with slightly increased incidence were observed in a number of other organs.

Liver

Several alterations were noted in the livers of tasimelteon-treated males and females. See the sponsor's summary table, below. Dose-related increases in the incidence and severity of centrilobular hepatocyte hypertrophy were reported in both sexes. The sponsor attributed the hypertrophy to "adaptive" drug-induced induction of hepatic enzymes. Increased incidences of bile duct hyperplasia were also reported in MD and

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HD animals; the sponsor considered this change a response to biliary excretion of the drug and/or its metabolites. In MD and HD males, increased incidences of regenerative hyperplasia, cystic degeneration, and centrilobular hepatocyte vacuolation were reported. In MD and HD females, pigment in the hepatocytes (identified as lipofuscin) was reported, as well as a low incidence of cystic degeneration in tasimelteon-treated females. Additionally, a few other findings of generally low incidence were observed, including: prominent increase in mitotic activity, focal fibrosis and portal bridging fibrosis, portal inflammation, and pigment in macrophages (see excerpts from the sponsor's summary table, below). The sponsor noted that reduced incidences of basophilic foci were reported for MD and HD females.

Summary of treatment related findings in the liver for all rats

Group/sex	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Dose (mg /kg/day)	**0**	**20**	**100**	**250**	**0**	**20**	**100**	**250**
Basophilic foci								
Minimal	8	3	7	8	13	11	20	2
Slight	3	4	4	2	20	14	2	3
Moderate	0	1	0	0	8	8	0	2
Total	11	8	11	10	41	33	22*	7*
Hepatocyte hypertrophy, centrilobular								
Slight	7	14	34	34	2	10	41	23
Moderate	1	0	19	27	0	0	13	20
Marked	0	0	0	0	0	0	0	2
Total	8	14	53*	61*	2	10*	54*	45*
Regenerative hyperplasia								
Minimal	0	0	1	3	1	0	0	2
Slight	2	5	7	3	5	6	6	5
Moderate	1	0	5	2	2	2	0	2
Marked	1	0	0	1	0	0	0	0
Total	4	5	13*	9	8	8	6	9
Bile duct hyperplasia								
Slight	1	4	10	6	4	7	8	6
Moderate	2	2	0	3	1	1	2	4
Marked	1	0	0	0	0	0	0	1
Total	4	6	10	9	5	8	10	11
Hepatocyte vacuolation, centrilobular								
Slight	3	7	10	11	1	2	2	1
Moderate	4	0	2	7	1	0	0	0
Total	7	7	12	18*	2	2	2	1
Cystic degeneration								
Minimal	14	14	13	12	2	6	5	6
Slight	5	9	18	15	1	2	1	2
Moderate	3	3	7	8	0	0	0	2
Marked	0	0	2	1	0	0	0	0
Total	22	26	40*	36*	3	8	6	10
Pigment in hepatocytes								
Minimal	0	0	0	0	0	0	1	4
Slight	0	0	0	0	0	0	8	15
Moderate	0	0	0	0	0	0	4	9
Marked	0	0	0	0	0	0	0	1
Total	0	0	0	0	0	0	13*	29*
Number of animals examined	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**

* Significant when compared to controls using a fishers two-tailed exact test. * - $p < 0.05$

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250

Tissue and Finding	Group/Sex: Number:	1F 65	2F 65	3F 65	4F 65
Liver	Number Examined:	65	65	65	65
Fibrosis, Portal Bridging		0	0	0	1
Focal Fibrosis		0	0	0	2
Inflammation, Portal		8	6	4	11
Pigment in Macrophages		3	4	4	7
Prominent Increase in Mitotic Activity		0	1	1	2

Kidney

Dose-related increased incidences of cortical tubular pigment (identified as lipofuscin) were reported in tasimelteon-treated females. The incidence of papillary/pelvic epithelial mineralization was reduced in treated females, compared to controls. See the sponsor's summary table, below. Although no findings were reported in males, a few low incidence alterations were observed (see excerpts from the sponsor's table, below). A slightly increased incidence of cortical cysts was observed in tasimelteon-treated males. In MD and HD males, cortical tubular basophilia and an increased incidence of tubular hypertrophy were observed. In HDM, increased incidences of pelvic dilatation (also in HDF) and mineralization (cortex, papilla, and/or pelvic/papillary epithelium) were also observed.

Summary of treatment related findings in the kidneys for all rats

Group/sex	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Dose (mg /kg/day)	**0**	**20**	**100**	**250**	**0**	**20**	**100**	**250**
Cortical tubular pigment								
Minimal	2	0	0	3	2	6	7	6
Slight	5	5	5	6	2	6	7	9
Moderate	0	0	1	0	1	0	1	1
Total	7	5	6	9	5	12	15*	16*
Mineralisation, papillary/pelvic epithelium								
Minimal	1	1	1	1	20	11	13	16
Slight	0	0	0	2	14	5	6	3
Moderate	0	0	0	0	7	8	0	0
Total	1	1	1	3	41	24*	19*	19*
Number of animals examined	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**

* Significant when compared to controls using a fishers two-tailed exact test. * - p <0.05

	ConM	LDM	MDM	HDM	ConF	LDF	MDF	HDF
Cortical cyst	4	8	10	7	1	2	1	1
Cortical tubular basophilia	0	0	1	3	1	0	0	1
Tubular hypertrophy	3	3	8	6	3	3	1	0
Pelvic dilatation	2	4	1	7	1	2	2	5
Mineralization, cortex	2	2	2	4				
Mineralization, Papilla	0	1	1	2				

Spleen
Hemosiderosis was observed with increased incidence in tasimelteon-treated males and HDF. See the summary table below.

	ConM	LDM	MDM	HDM	ConF	LDF	MDF	HDF
Hemosiderosis	5	11	6	15	33	29	26	41

Ovaries
Increased incidences of cysts were reported in MD and HD females. See the sponsor's summary table, below. A few stromal tumors were observed in MDF and HDF; interstitial cell hyperplasia and Sertoli cell hyperplasia were slightly increased in incidence. In addition, absence of corpora lutea and focal fibrosis were observed (see excerpts from the sponsor's summary table, below).

Summary of treatment related findings in the ovaries for all rats

Group/sex **Dose (mg /kg/day)**	**1F** **0**	**2F** **20**	**3F** **100**	**4F** **250**
Cyst(s)				
Slight	2	1	5	6
Moderate	2	4	4	5
Marked	0	0	1	0
Total	4	5	10	11
Number of animals examined	**64**	**65**	**64**	**63**

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250

Tissue and Finding	Group/Sex:	1F	2F	3F	4F
	Number:	65	65	65	65
Absent Corpora Lutea		33	28	36	39
Interstitial Cell Hyperplasia		33	38	42	33
Sertoli Cell Hyperplasia		30	23	26	37
Focal Fibrosis		0	1	1	1

Uterus

In addition to uterine tumors, glandular dilatation and hyperplasia were observed in MD and HD females (see excerpt from the sponsor's summary table, below).

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	20	100	250

Tissue and Finding	Group/Sex:	1F	2F	3F	4F
	Number:	65	65	65	65
Uterus	Number Examined:	64	65	64	64
Glandular Dilatation		14	14	21	22
Glandular Hyperplasia		1	1	2	2

Uterine Cervix

Increased incidences of epithelial hyperplasia and epithelial keratinization were reported in HDF. Also, the incidence of mural/stromal hypertrophy/hyperplasia was slightly increased in HDF (see excerpt from the sponsor's summary table, below).

Summary of treatment related findings in the uterine cervix for all rats

Group/sex		**1F**	**2F**	**3F**	**4F**
Dose (mg /kg/day)		**0**	**20**	**100**	**250**
Epithelial hyperplasia					
	Slight	9	10	7	19
	Moderate	0	1	3	1
	Total	9	11	10	20*
Epithelial keratinisation					
	Minimal	0	2	2	2
	Slight	3	3	4	9
	Moderate	0	0	0	1
	Total	3	5	6	12*
Number of animals examined		**64**	**65**	**64**	**64**

* Significant when compared to controls using a fishers two-tailed exact test. * - $p < 0.05$

Group	:	1	2	3	4			
Compound	:	Control	VEC-162	VEC-162	VEC-162			
Dose (mg/kg/day)	:	0	20	100	250			
				Group/Sex:	1F	2F	3F	4F
Tissue and Finding				Number:	65	65	65	65
Uterine cervix				Number Examined:	64	65	64	64
Mural / Stromal Hypertrophy/ Hyperplasia					14	15	9	18

Mammary Gland

Although some neoplastic changes were observed in females (adenomas [HD], adenocarcinomas [MD and HD]), drug-related increases in the incidences of non-neoplastic alterations were not clearly observed in females; ductular hyperplasia was detected only in one HDF. However, the incidences of ductular dilatation and galactoceles were slightly increased in tasimelteon-treated males and in MD and HD males, respectively. See excerpts from the sponsor's table, below.

Group	:	1	2	3	4			
Compound	:	Control	VEC-162	VEC-162	VEC-162			
Dose (mg/kg/day)	:	0	20	100	250			
				Group/Sex:	1M	2M	3M	4M
Tissue and Finding				Number:	65	65	65	65
Mammary				Number Examined:	65	65	65	65
Ductular Dilatation					2	5	9	5
Galactocele(s)					1	0	2	2

Testes

There was only a suggestion that interstitial Leydig cell adenomas were increased (HDM and possibly MDM); however, the incidence of interstitial cell hyperplasia was doubled at MD and HD. There was a very slight increase in arteritis/periarteritis. See excerpt from the sponsor's table, below.

Group	:	1	2	3	4			
Compound	:	Control	VEC-162	VEC-162	VEC-162			
Dose (mg/kg/day)	:	0	20	100	250			
				Group/Sex:	1M	2M	3M	4M
Tissue and Finding				Number:	65	65	65	65
Testes				Number Examined:	65	65	65	65
B-Interstitial (Leydig) Cell Adenoma					3	1	4	6
Interstitial Cell Hyperplasia					4	4	8	8

Pituitary

Although there was only a suggestion of an increase above the spontaneous incidence of adenomas in the pituitary pars distalis in males, the incidence of hyperplasia was clearly increased at HD. Hypertrophy and angiectasis was slightly increased in

tasimelteon-treated males. Vacuolated cells in the pars distalis were slightly increased in HDM. Drug-related pituitary changes were not observed in females.

Pituitary	Number Examined:	63	65	65	64
Angiectasis		0	2	1	2
Hyperplasia - Pars Distalis, Focal		2	0	1	8
Hypertrophy - Pars Distalis, Focal		4	8	12	6
Vacuolated Cells - Pars Distalis, Focal		7	5	7	9

Other organs

A few tissues showed limited, low incidence changes of unclear toxicological importance (see table below for details). Changes were suggested for the eyes, lung, heart, urinary bladder, stomach, pancreas, thyroid, adrenal, bone marrow, Peyer's patches, lymph nodes, thymus, skin and integumentary tissues, prostate, and epididymides.

Organ/Finding	ConM	LDM	MDM	HDM	ConF	LDF	MDF	HDF
Eyes								
Retinal atrophy	0	1	4	1	1	7	4	3
Lung								
Alveolar epithelial hyperplasia	3	2	2	4	0	1	1	5
Pigmented alveolar macrophages	-	-	-	-	0	0	1	3
Peribronchiolar lymphoid aggregates	4	5	7	8	8	5	8	16
Heart								
Vascular Mineralization	0	3	2	3	-	-	-	-
Urinary Bladder								
Luminal Dilatation	4	4	2	7	2	5	1	3
Stomach								
Dilated glands	19	19	16	21	20	28	23	29
Submucosal inflammation, glandular	7	10	10	3	1	3	4	4
Pancreas								
Acinar cell vacuolation	0	1	1	5	0	0	0	1
Islet cell hyperplasia	1	0	4	3	0	1	1	1
ISLET CELL ADENOMA	1	5	5	2	2	2	0	0
ISLET CELL CARCINOMA	1	1	2	0	-	-	-	-
Thyroid								
Follicular Cell hypertrophy	1	3	0	5	-	-	-	-
Follicular cell atrophy	-	-	-	-	0	0	3	3
Follicular cell cyst	-	-	-	-	0	0	2	3

Organ/Finding	ConM	LDM	MDM	HDM	ConF	LDF	MDF	HDF
Adrenal								
Cortical/Cystic/Hemorrhagic Degeneration	7	14	18	10	51	57	52	50
Sinusoidal Dilatation/Congestion	5	9	14	11	32	38	42	40
Sternum and Marrow								
Myeloid hyperplasia, marrow	-	-	-	-	1	0	0	6
Femur and joint								
Myeloid hyperplasia, marrow	1	1	1	1	7	1	9	13
Ileum/Peyer's patches								
GALT increased cellularity	0	4	2	1	0	0	0	2
Mesenteric LN								
Dilated/Cystic Sinuses	4	11	15	10	-	-	-	-
Mandibular LN								
Dilated/Cystic Sinuses	4	2	6	11	3	4	6	4
Paracortex, increased cellularity	25	19	18	16	6	6	3	9
Thymus								
Epithelial hyperplasia	1	1	3	2	12	11	14	19
Skin								
Areas of Prominent Collagen	2	1	4	4	-	-	-	-
Epidermal ulceration	0	0	0	3	0	1	2	2
Pinnae								
Epidermal hyperplasia	0	0	2	1	-	-	-	-
Epidermal ulceration	0	0	1	0	1	0	2	0
Tail								
Epidermal hyperplasia	0	0	1	1	-	-	-	-
Lacrimal gland								
Acinar atrophy	2	1	2	5	3	3	1	2
Harderian gland								
Acinar atrophy	1	2	1	4	1	0	2	0
Porphyrin concretions	11	15	19	16	9	12	11	11
Clitoral Gland								
Lymphoid aggregates					14	26	22	22
Prostate								
Acinar hyperplasia, focal	0	0	3	1				
Inflammation	4	4	2	7				
Epididymides								
Inflammation	0	3	1	4				

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Toxicokinetics [Week 4 and Week 26, as well as during Week 96 from 3/sex/gp from main study; see the sponsor's summary table below]

Week No	Group/Sex	Time of sampling (hours after dosing) 1	2	4	8	12	24
		Animal Number					
4	1M	66-68	69-71	72-74	66-68	67-71	72-74
	1F	366-368	369, 371, 375	372-374	366-368	369, 371, 375	372-374
	2M	141-143	144-146	147-149	141-143	144-146	147-149
	2F	441-443	444-446	447-449	441-443	444-446	447-449
	3M	216-218	219-221	222-224	216-218	219-221	222-224
	3F	516-518	519-521	522-524	516-518	519-521	522-524
	4M	291-293	294-296	297-299	291-293	294-296	297-299
	4F	591-593	594-596	597-599	591-593	594-596	597-599

Week No	Group/Sex	Time of sampling (hours after dosing) 1	2	4	8	12	24
		Animal Number					
26	1M	66-68	69-71	72-74	66-68	69-71	72-74
	1F	366, 368	369, 371, 375	372-374	366, 368	369, 371, 375	372-374
	2M	141-143	144-146	147-149	141-143	144-146	147-149
	2F	441-443	444-446	447-449	441-443	444-446	447-449
	3M	216-218	219-221	222-224	216-218	219-221	222-224
	3F	516-518	519-521	522-524	516-518	519-521	522-524
	4M	291-293	294-296	291-293	294-296	291-293#	294-296#
	4F	591-593	594-596	597-599	591-593	594-596	597-599

Week No	Group/Sex	Time of sampling (hours after dosing) 0.5	2
		Animal Number	
96	1M	10, 11, 13	14, 18, 19
	1F	301, 302, 303	304, 305, 306
	2M	76, 78, 81	82, 84, 87
	2F	378, 379, 388	385, 386, 387
	3M	152, 158, 161	164, 165, 166
	3F	453, 456, 459	460, 461, 462
	4M	232, 233, 236	237, 238, 240
	4F	526, 531, 535	537, 538, 542

The blood samples from Week 96 were not analyzed but were stored frozen for potential future analysis. Throughout the study, plasma samples taken from control animals demonstrated tasimelteon exposures above the LLOQ (0.2 to 10.6 ng/mL); while suggesting that there was some contamination, these levels are well below exposures achieved in the tasimelteon-treated animals (at least 100-fold). Generally, systemic exposure to tasimelteon increased over the dose range, in a less than dose-proportional fashion (with the exception of week 4 AUCs). Week 26 exposures were similar to Week 4 exposures (except the AUC increased between Week 4 and Week 26 in LDM). A sex difference in exposure was demonstrated (females > male). See the sponsor's summary exposure data, below.

Week 4

Males

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (hours^{-1})	t½ (hours)
20	998	1	0.437	3650	a	-
100	4830	1	2.25	28400	a	-
250	8810	4	17.9	59700	a	-

Females

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (hours^{-1})	t½ (hours)
20	1840	2	1.78	7320	a	-
100	6570	4	6.65	45000	0.3733	1.9
250	17200	4	62.2	103000	a	-

Week 26

Males

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (hours^{-1})	t½ (hours)
20	2830	1	4.57	6520	a	-
100	5060	2	2.36	38800	a	-
250	9890	2	70.8	52500	0.2467	2.8

Females

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (hours^{-1})	t½ (hours)
20	1640	2	1.57	9900	a	-
100	7490	2	2.69	42000	0.3951	1.8
250	13400	4	6.88	101000	0.4310	1.6

a Terminal rate constant could not be adequately estimated

(* Note: Livers from the satellite TK animals were harvested and snap frozen in liquid nitrogen. The tissues were then shipped on dry ice to (b) (4) The results of any analyses performed on these tissues were not reported here.)

Dosing Solution Analysis [Weeks 1, 13, 26, 39, 52, 65, 78, 91 and 103]

Dosing formulations were prepared each week and stored refrigerated (2-8°C) and protected from light. Previously, homogeneity and stability were demonstrated at 7.5 and 100 mg/mL for 24 hours at ambient temperature (nominally 21°C) and for 15 days refrigerated (nominally 2-8°C). The dosing formulations prepared for Weeks 1, 13, 26, 39, 52, 65, 78, 91 and 103 of treatment were analyze. The mean concentrations were within ± 10% of the nominal values, with individual results within 3% of the mean value.

Interspecies Parent and Metabolite Comparison

For reference, the sponsor provided the following summary table of estimated metabolite exposures in the rat carcinogenicity study (see sponsor's Table 18 from the Nonclinical Toxicology Written Summary, following.)

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Table 18: Tasimelteon metabolite levels in rats estimated from tasimelteon plasma levels measured at Week 26 in Study TAJ0001 using the metabolite/tasimelteon AUC ratios determined in Study TAJ0014

		AUC_{0-24} (ng.h/mL)								
Rat		Tasimelteon	M9 ratio[a]	M9	M12 ratio[a]	M12	M13 ratio[a]	M13	M3 ratio[a]	M3
20[b]	M	6,520	0.25	1,630	2.14	13,953	0.03	196	0.29	1,891
	F	9,900	0.28	2,772	4.21	41,679	0.01	99	0.25	2,475
100	M	38,800	0.32	12,416	2.51	97,388	0.03	1,164	0.58	22,504
	F	42,000	0.12	5,040	2.45	102,900	0.02	840	0.26	10,920
250	M	52,500	0.26	13,650	2.08	109,200	0.06	3,150	0.73	38,325
	F	101,000	0.15	15,150	1.75	176,750	0.02	2,020	0.43	43,430
Human[c] 20 mg QD		363	--	423	--	619	--	353	--	192

[a] Metabolite/tasimelteon AUC ratios from Study TAJ0014 at comparable doses (corrected for molecular weights).
[b] Metabolite/tasimelteon AUC ratios for 20 mg/kg were those determined for 25 mg/kg doses from Study TAJ0014.
[c] Data for tasimelteon and M9, M12, and M13 are from Study VP-VEC-162-1110: An Open-Label, Single Sequence Study to Assess the Effect of Multiple Doses of Tasimelteon on the Cytochrome P450 3A4 and 2C8 Enzymes using Midazolam and Rosiglitazone as Substrates. Data for M3 are from group 2 in Study VP-VEC-162-1107: An open-label, single-dose, parallel group study to assess the effects of smoking status, age and body size on the pharmacokinetics, safety, and tolerability of tasimelteon in healthy volunteers.

Study title: VEC-162: CARCINOGENICITY STUDY BY ORAL GAVAGE ADMINISTRATION TO CD-1 MICE FOR 104 WEEKS	
Study no.:	TAJ0002
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/14/06
GLP compliance:	Yes (b) (4)
QA statement:	Yes,
Drug, lot #, and % purity:	VEC-162, batches 800156790, 800156800, and 800167150, 99.7-100.7% pure
CAC concurrence:	Yes (see ExecCAC minutes dated 8/23/99). Recommended doses were 30, 100, and 300 mg/kg.

Key Study Findings

No neoplasms reported by the sponsor.

Adequacy of Carcinogenicity Study

Overall, the study was adequate. The study used the doses recommended by the ExecCAC (see minutes dated 8/23/99).

Appropriateness of Test Models

The mouse is a standard species used for 2-year carcinogenicity bioassays.

Evaluation of Tumor Findings

None.

Methods (See summary design table from the sponsor's submission, below.)	
Frequency of dosing:	QD
Dose volume:	4 mL/kg
Route of administration:	PO, oral gavage
Formulation/Vehicle:	100% polyethylene glycol-400 (PEG400)
Basis of dose selection:	MTD. In a 3-month dose-ranging study, a single dose of 800 mg/kg caused mortality following severe clinical signs including labored breathing; the dose was lowered to 600 mg/kg without further mortality. At 600 and 400 mg/kg, clinical signs of hypoactivity, prostration, and labored breathing were observed in addition to centrilobular hypertrophy of the liver. Based on lethality at 800 mg/kg and similar signs at 600 mg/kg, a HD of 300 mg/kg was recommended by the ExecCAC. The remaining doses were placed at appropriate intervals, with the LD expected to provide a plasma AUC equal to that anticipated in humans at a dose of 50 mg.
Species/Strain:	Crl: CD-1™ (ICR) BR mice (b) (4)
Age:	37 to 43 days at initiation
Weight:	M: 22 to 42 g; F: 20 to 33 g
Animal housing:	3/sex/cage, unless reduced by mortality or isolation
Satellite groups:	TK (see **Toxicokinetics** for details)
Deviation from study protocol:	The sponsor noted 4 deviations that were not considered to have had an impact on the integrity or validity of the study.

Group	Treatment	Dose# (mg/kg/day)	Main study No. of animals Male	Female	Animal numbers Male	Female	Cage numbers Male	Female
1	Control	0	66	66	1-66	421-486	1-22	141-162
2	VEC-162	30	66	66	106-171	526-591	36-57	176-197
3	VEC-162	100	66	66	211-276	631-696	71-92	211-232
4	VEC-162	300	66	66	316-381	736-801	106-127	246-267

Group	Treatment	Dose# (mg/kg/day)	Satellite study† No. of animals Male	Female	Animal numbers Male	Female	Cage numbers Male	Female
1	Control	0	39	39	67-105	487-525	23-35	163-175
2	VEC-162	30	39	39	172-210	592-630	58-70	198-210
3	VEC-162	100	39	39	277-315	697-735	93-105	233-245
4	VEC-162	300	39	39	382-420	802-840	128-140	268-280

Expressed in terms of test material as supplied.
† Satellite animals used for Toxicokinetics sampling only (culled after sampling in Week 26).

Observations and Results

Mortality [2x/day]

There was no clear drug-related effect on mortality. See the sponsor's summary table and Figure 1, below. Histopathological findings reported as factors contributing to death included: bronchioalveolar carcinoma (tasimelteon-treated females) and lung lesions, laryngeal/tracheal lesions (HD animals), sarcoma NOS (tasimelteon-treated females), and skin/ear lesions (HDM).

Group distribution of mortality

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	30	100	300	0	30	100	300
Premature deaths	30	38	32	33	37	41	47	38
Percentage survival	55	42	52	50	44	38	30	42

FIGURE 1

Percentage survival versus period of treatment

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	30	100	300

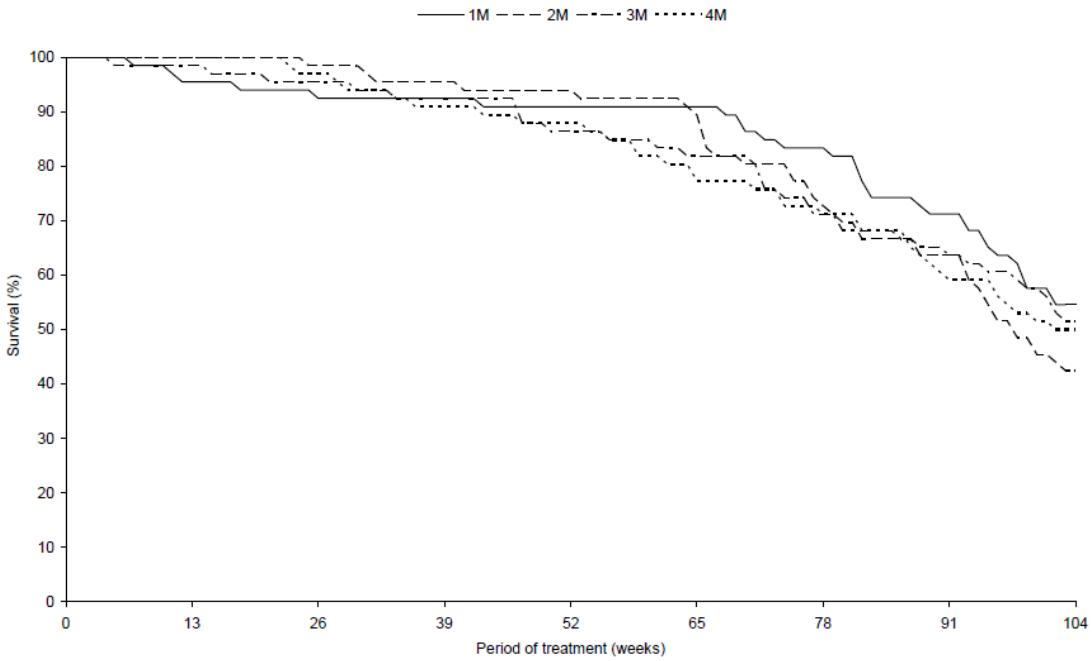
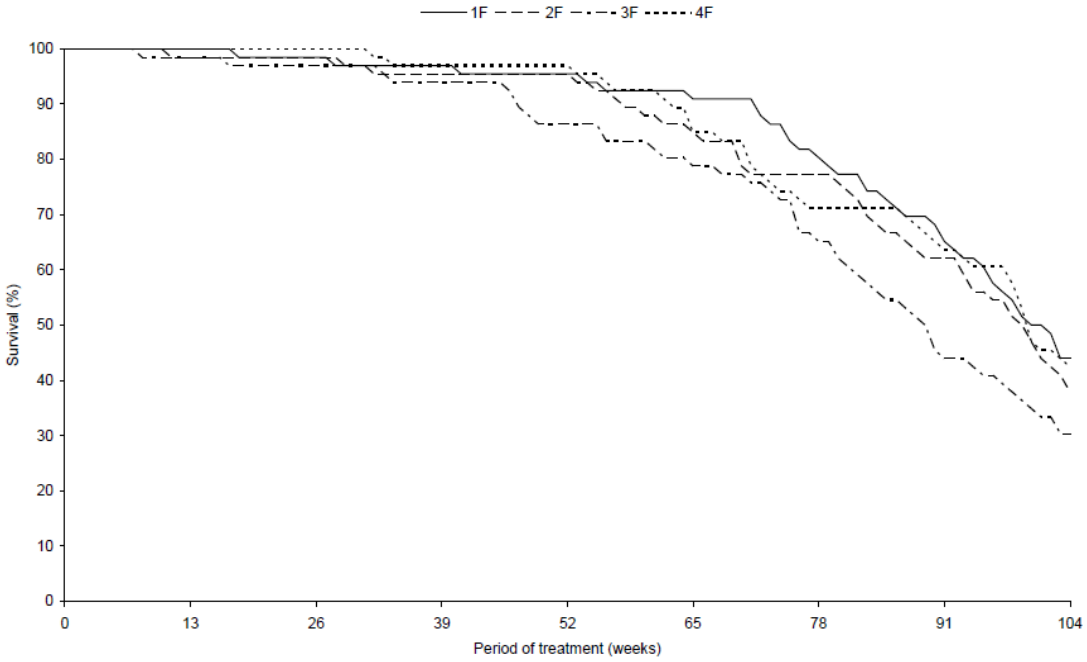


FIGURE 1 - continued

Percentage survival versus period of treatment

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	30	100	300



Clinical Signs [2x/daily, with detailed physical exams weekly, and detailed observations recorded daily during Week 1, twice weekly during Weeks 2-4, once weekly during Weeks 5-13, biweekly during Weeks 14-52, and once every 4 weeks thereafter]

Observations were performed with following timing on the specified days: immediately before dosing, immediately after dosing on return of the animal to its cage, on completion of dosing of each group, between one and two hours after completion of dosing of all groups, and as late as possible in the working day.

The sponsor reported few clinical signs. "Underactive" was reported in most HD animals after dosing, at 30 to 120 minutes after dosing; this occasionally lasted until the end of the workday. MD (beginning week 50) and LD (beginning Week 52) animals were also reported as "underactive" occasionally. Partial closure of the eyelids was reported intermittently in MD and HD animals beginning in Week 26 and was most consistent during Weeks 50 to 52; it was observed at 1 or 2 hours after dosing and sometimes lasted until the end of the workday. Dark eye(s) were observed with slightly increased incidence in tasimelteon-treated females.

No drug-related effects on palpable swellings were reported. See the sponsor's summary Table 3 below for details.

TABLE 3

Palpable swellings - group distribution, multiplicity and mean time of onset+

Group :	1	2	3	4
Compound :	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day) :	0	30	100	300

Group /Sex	Multiplicity∅ 0	1	2	3	4 or more	Number of animals with swellings	Total number of swellings	Mean time of onset*
1M	49	11	5	0	1	17	26	71
2M	53	10	2	1	0	13	17	80
3M	54	11	0	1	0	12	14	64
4M	55	8	2	0	1	11	16	70
1F	55	6	2	3	0	11	19	67
2F	58	7	1	0	0	8	9	63
3F	54	11	0	0	1	12	15	75
4F	59	5	2	0	0	7	9	79

\+ Including swellings which regressed or were not positively identified at *post mortem* examination
∅ Expressed as number of animals bearing the indicated number of swellings
* In weeks to onset of first recorded swelling including those found at necropsy examination

Body Weights [Week-1, Day 1, weekly for 12 weeks, every 4 weeks thereafter]

Body weight gain was reduced in HD animals (males [ss] > females [nss]) compared to controls. This was most apparent between Weeks 36 and 80; see the sponsor's summary table and Figure 2, below. Average body weight was reduced approximately

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10% in HDM at the end of the study. Overall, LDF and MDF tended to gain slightly more weight than the respective control.

Group mean bodyweight gain (g)

Group and sex Dose (mg/kg/day)	1M 0	2M 30	3M 100	4M 300	1F 0	2F 30	3F 100	4F 300
Week 0-36	13.8	14.3	14.2	12.3	13.0	13.2	13.5	12.5
As a % of Group 1	-	104	103	89	-	102	104	96
Week 36-80	2.8	3.0	2.7	1.1**	4.9	5.7	5.0	3.5
As a % of Group 1	-	107	96	39	-	116	102	71
Week 80-104	-3.3	-2.1	-4.4	-2.2	-4.0	-3.6	-4.6	-2.0
As a % of Group 1	-	-	-	-	-	-	-	-
Week 0-104	14.9	13.9	12.7	11.1**	13.7	14.6	15.2	14.0
As a % of Group 1	-	93	85	74	-	107	111	102

Significant when compared to Group 1: * $p<0.05$; ** $p<0.01$

FIGURE 2

Bodyweight - group mean values versus period of treatment - males

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	30	100	300

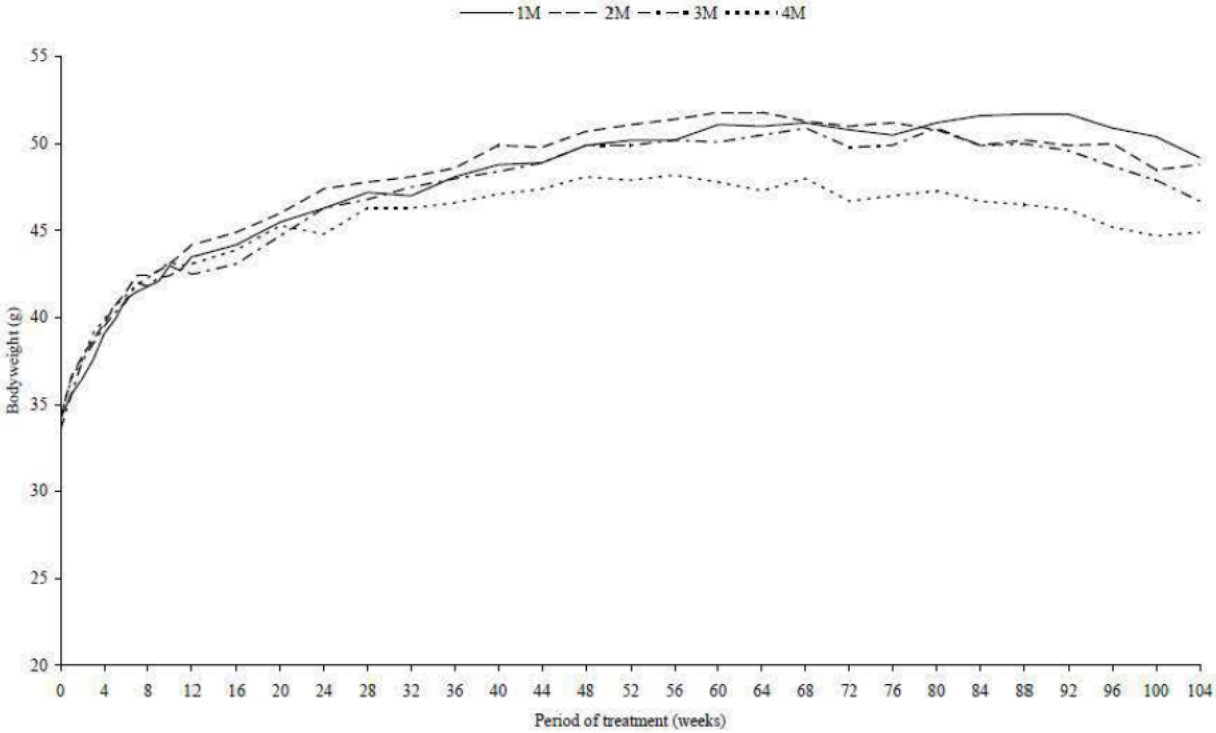
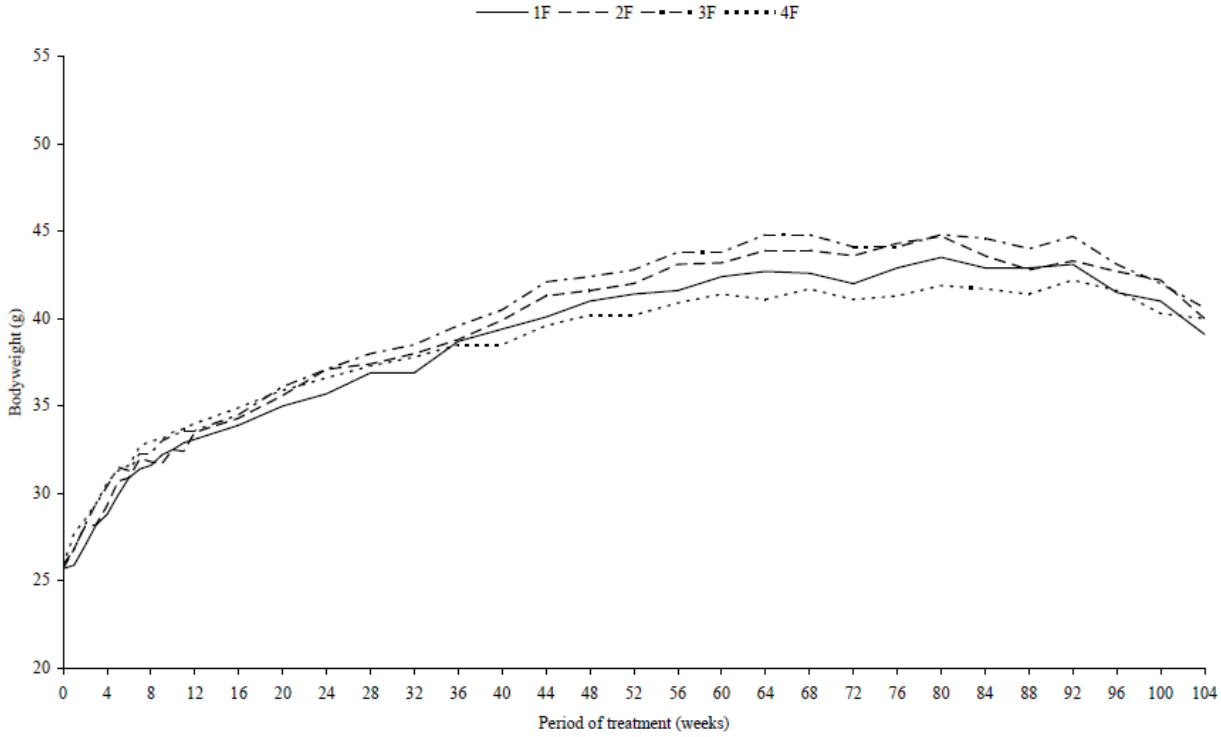


FIGURE 2 - continued

Bodyweight - group mean values versus period of treatment - females

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	30	100	300



Food Consumption [Weekly for 12 weeks, every 4 weeks thereafter]

Food consumption was reduced in HD animals beginning approximately Week 16 (slightly earlier in females) compared to controls. See the sponsor's summary table, below.

Group mean food consumption (g/animal/week)

Group and sex Dose (mg/kg/day)	1M 0	2M 30	3M 100	4M 300	1F 0	2F 30	3F 100	4F 300
Week 1-16	37	38	36	37	34	34	34	32
As a % of Group 1	-	103	97	100	-	100	100	94
Week 16-104	38	38	36	32	34	34	34	30
As a % of Group 1	-	100	95	84	-	100	100	88
Week 1-104	38	38	36	33	34	34	34	31
As a % of Group 1	-	100	95	87	-	100	100	91

Hematology [Week 52, Week 104]

Blood samples were obtained from the orbital sinus of 20 main study animals/sex/group at Week 52, and the remaining animals (not sampled at Week 52 were sampled at Week 104. A limited list of parameters were evaluated, including: hemoglobin, hematocrit, erythrocyte count (RBC), total leucocyte count (WBC), neutrophils (N), lymphocytes (L), eosinophils (E), basophils (B), monocytes (M), large unstained cells (LUC), and morphology.

The sponsor reported slight dose-related reductions (~4-6%) in red cell parameters in MDF, HDF and/or HDM; hemoglobin and hematocrit (Week 52 only), MCH (both times, HDF), MCHC (both times, MDF and HDF), and MCV (Week 104, HDF and HDM). Platelet counts were increased (~5-15%) in HDF at Week 52 and in MDM, HDM and HDF at Week 104.

Organ Weights

Terminal body weights were reduced approximately 9% in HDM; average terminal body weights of all other groups were within 5% of their respective controls. The sponsor identified alterations in liver, kidney, and heart, and stated that apparent alterations in brain and adrenal were considered incidental. Average relative liver weights were increased 14% in HDM. Relative kidney and heart weights were reduced 10-17% and 7-10%, respectively, in MDM, HDM, and HDF. Brain weight was slightly reduced in HD animals (~4%). Absolute adrenal weight was reduced in HDM (16%). The relative adrenal weight reduction would be approximately 7% (if adjusted by the 9% reduced body weight); also, if adjusted for the slight increase in average body weight (3%) in HDF relative adrenal weight would be similarly reduced (~8%).

A few other noted differences were of unclear toxicologic concern. Average spleen weight was increased (~20%) in HDM. Average relative thymus weight was reduced approximately 10 to 20% in HDF. Average uterus weights were reduced approximately 10 to 15% at MD and HD. Average ovary weights were highly variable, but an increase of 81% was observed at HD (and LD).

Gross Pathology

The sponsor reported no drug-related macroscopic changes; however, there were a few changes of low or increased incidence. Changes were observed in liver, kidneys, ureters, heart, brain, thymus, reproductive organs, trachea, lung, eye, GI tract, and adipose tissue. In addition to these changes, a number of lymph nodes were noted to be enlarged in tasimelteon-treated animals.

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Group	1	2	3	4
Compound	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	0	30	100	300

Tissue and finding / Group/sex: Number Examined:	1M 66	2M 66	3M 66	4M 66	1F 66	2F 66	3F 66	4F 66
Liver								
Irregular surface	1	1	0	1	1	0	4	3
Kidneys								
Depression(s)	10	6	11	8	0	3	2	3
Enlarged	4	8	3	0	0	2	2	1
Ureters								
Thickened	1	1	1	2	4	2	8	1
Thymus								
Dark	0	0	0	0	0	1	0	1
Dark area(s)	0	1	0	0	3	1	2	5
Uterus								
Abnormal contents	-	-	-	-	1	0	4	3
Elongated	-	-	-	-	6	8	8	8
Testes								
Dark	0	2	1	4	-	-	-	-
Dark area(s)	2	0	1	0	-	-	-	-
Flaccid	11	20	14	11	-	-	-	-
Small	1	10	5	2	-	-	-	-
Seminal vesicles								
Small	0	1	1	3	-	-	-	-
Preputial glands								
Cyst(s)	8	10	10	14	-	-	-	-
Cystic	0	1	1	0	-	-	-	-
Cystic enlargement	1	0	1	0	-	-	-	-
Trachea								
Abnormal contents	0	0	0	3	0	1	3	2
Lungs + bronchi								
Dark area(s)	2	1	1	4	1	4	1	4
Optic nerves								
Thin	1	1	3	5	5	2	2	1
Eyes								
Prominent	2	0	2	7	4	3	1	0
Stomach								
Corpus dark area(s)	0	1	0	0	0	0	0	2
Distended	0	2	4	4	1	2	5	5
Mass(es)	1	1	3	1	1	0	2	5
Pale area(s)	0	0	0	0	0	0	0	2
General comments								
Abnormal contents gi tract	3	8	9	8	15	13	10	9
Pallor of internal tissues	1	1	0	0	1	4	5	3
Adipose tissue								
Dark	0	0	0	0	1	0	3	2
Dark area(s)	0	0	0	0	0	0	0	1
Mass(es)	2	1	2	0	2	5	4	3
Pinnae								
Thickened	0	0	1	3	0	0	0	0

Histopathology

Adequate Battery	**Yes (see Appendix 1)**
Separate, Signed Report	**Yes (limited version)**
Peer Review	**Yes, from (b) (4) and a consultant, not identified**

Neoplastic

The sponsor reported no drug-related neoplasias. The FDA statistical reviewer did not report any statistically significant incidences of neoplasms; his assessment is included the following excerpts from Table A.2.2, below.

There were a few low incidence findings of unclear toxicological importance. Lymphoma, leukemia, and sarcoma metastases appeared in numerous tissues in tasimelteon-treated animals. Skeletal muscle and skin (all sarcoma NOS occurred in early decedents), GI tract (stomach and intestines), mammary gland (most acanthomas occurred in early decedents), and reproductive organs showed a few neoplasms.

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Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	30	100	300

Tissue and Finding	Group/Sex: Number:	1M 66	2M 66	3M 66	4M 66	1F 66	2F 66	3F 66	4F 66
Skeletal Muscle	Number Examined:	66	66	66	66	66	66	66	66
M-Fibrosarcoma		0	0	0	0	0	1	0	0
M-Sarcoma NOS		0	0	0	0	0	1	1	2
Skin	Number Examined:	66	66	66	66	66	66	66	66
M-Sarcoma NOS		0	0	0	0	0	0	0	1
Stomach	Number Examined:	66	66	66	66	66	66	66	66
B-Adenoma		0	0	1	0	0	0	0	0
B-Squamous Cell Papilloma		0	0	0	0	0	0	1	0
M-Squamous Cell Carcinoma		0	0	0	0	0	0	0	1
Duodenum	Number Examined:	66	65	65	66	66	66	65	65
B-Adenoma		0	0	0	2	0	0	0	0
Colon	Number Examined:	66	66	66	66	66	66	65	65
B-Adenoma		0	0	0	1	0	0	0	0
Jejunum	Number Examined:	66	65	65	66	64	66	64	65
B-Adenoma		0	0	0	0	0	0	0	1
M-Adenocarcinoma		0	0	0	1	0	0	0	0
Caecum	Number Examined:	66	66	64	65	65	66	64	65
B-Adenoma		0	0	0	1	0	0	0	0
[Intestines									
B-adenoma		**0**	**0**	**0**	**4**	**0**	**0**	**0**	**1**
M-adenocarcinoma		**2**	**0**	**0**	**2**	**0**	**0**	**0**	**0**
Combined		**2**	**0**	**0**	**6**	**0**	**0**	**0**	**1]**
Mammary	Number Examined:	66	66	66	66	66	66	66	66
M-Mammary Adenoacanthoma		0	0	0	0	1	1	3	1
Epididymides	Number Examined:	66	66	66	66	-	-	-	-
B-Interstitial (Leydig) Adenoma		0	0	1	0	-	-	-	-
Testes	Number Examined:	66	66	66	66	-	-	-	-
B-Interstitial (Leydig) Cell Adenoma		1	1	3	2	-	-	-	-
Uterus	Number Examined:	-	-	-	-	66	66	66	66
B-Leiomyoma		-	-	-	-	1	1	1	2
Uterine cervix	Number Examined:	-	-	-	-	66	64	65	66
B-Endometrial Polyp		-	-	-	-	0	0	1	2
M-Endometrial Stromal Sarcoma		-	-	-	-	0	1	0	0

Table 4. Potentially Statistically Significant Neoplasms in Mice

Sex/ organ/ tumor	Incidence Veh	Low	Med	High	Significance Levels ptrend	phigh vsVeh	pmed vsVeh	plow vsVeh
Male Mice								
Caecum+Colon+Duodenum								
# Evaluated	66	66	66	66				
Adj. # at Risk	50.1	45.8	46.5	45.1				
Adenoma	0	0	0	3	.0134	.1025	.	.
Caecum+Colon+Duodenum+Jejunum								
# Evaluated	66	66	66	66				
Adj. # at Risk	50.6	45.8	46.5	45.4				
Adenoma/Adenocarcinoma	2	0	0	5	.0114	.1764	1	1
DUODENUM								
# Evaluated	66	65	65	66				
Adj. # at Risk	50.1	44.9	46.5	45.1				
ADENOMA	0	0	0	2	.0582	.2217	.	.
Female Mice								
UTERINE CERVIX								
# Evaluated	66	64	65	66				
Adj. # at Risk	48.6	44.3	39.8	48.1				
ENDOMETRIAL POLYP	0	0	1	2	.0652	.2474	.4483	.

Non Neoplastic

The sponsor reported drug-related histopathological changes in the liver at ≥MD in males and at HD in both sexes. See the sponsor's summary table for the incidence and severity of hepatocyte hypertrophy observed, below. Also, a slightly increased incidence of necrosis was observed (see excerpt from the sponsor's table).

Summary of treatment related findings in the livers for all mice

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg /kg/day)	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Hepatocyte hypertrophy, centrilobular								
Minimal	17	16	26	30	5	3	6	19
Slight	1	2	2	11	0	1	1	3
Total	18	18	28	*41	5	4	7	*22
Number of animals examined	**66**	**66**	**66**	**66**	**66**	**66**	**66**	**66**

* Significant when compared to controls using a fishers two-tailed exact test. * - p <0.05

Group	:	1	2	3	4				
Compound	:	Control	VEC-162	VEC-162	VEC-162				
Dose (mg/kg/day)	:	0	30	100	300				
				Group/Sex:	1M	2M	3M	4M	1F	2F	3F	4F
Tissue and Finding				Number:	66	66	66	66	66	66	66	66
Liver				Number Examined:	66	66	66	66	66	66	66	66
Hepatocyte Necrosis, Focal					2	1	3	5	4	4	5	6
Necrosis/Fibrosis/Haemorrhage					0	0	0	0	1	3	0	3

In addition to the liver change noted by the sponsor, there were a few low or slightly increased incidence observations of unclear toxicological importance. There was minimal evidence of changes in kidney, urinary bladder, GI tract, immune and hematologic tissues, respiratory tract, integument, Harderian gland, optic nerve, and reproductive tissues.

Group	:	1	2	3	4							
Compound	:	Control	VEC-162	VEC-162	VEC-162							
Dose (mg/kg/day)	:	0	30	100	300							
				Group/Sex:	1M	2M	3M	4M	1F	2F	3F	4F
Tissue and Finding				Number:	66	66	66	66	66	66	66	66
Kidneys				Number Examined:	66	66	66	66	66	66	66	66
Cortical Tubular Dilatation					6	12	11	6	6	7	3	9
Cortical Tubular Pigment					1	0	0	1	2	2	2	3
Cortical Tubules with Hyaline Droplets					0	4	2	1	4	8	6	5
Corticomedullary Tubular Dilatation					0	0	0	1	0	1	1	2
Papillary Necrosis				0	0	1	0	0	0	0	1	
Urinary Bladder				Number Examined:	66	64	66	66	66	66	65	66
Cystitis					0	0	0	1	0	0	0	0
Lymphoid Aggregates					14	10	16	10	20	20	28	27

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Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	30	100	300

Tissue and Finding	Group/Sex: Number:	1M 66	2M 66	3M 66	4M 66	1F 66	2F 66	3F 66	4F 66
Stomach	Number Examined:	66	66	66	66	66	66	66	66
Adenomatous Hyperplasia - Glandular Region		5	5	3	7	9	4	2	0
Epithelial Hyperplasia - Glandular Region		1	0	1	0	0	0	1	2
Colon	Number Examined:	66	66	66	66	66	66	65	65
Mucosal Hyperplasia		0	1	4	1	1	0	0	0
Spleen	Number Examined:	66	66	66	66	66	66	66	66
Haemosiderosis		14	21	25	23	26	25	30	28
White Pulp - Increased Cellularity		0	2	0	1	1	2	3	3
Thymus	Number Examined:	61	57	57	56	59	59	63	63
Lymphoid Hyperplasia		0	0	0	1	1	5	3	2
LN Mandibular	Number Examined:	66	65	64	62	65	64	63	63
Sinus Histiocytosis		2	1	4	4	0	3	3	2
Sternum + Marrow	Number Examined:	66	66	66	66	66	65	66	66
Marrow - Increased Granulopoiesis		5	8	6	9	8	9	4	3
Femur inc. Joint	Number Examined:	66	66	66	66	66	66	66	66
Marrow - Fat Replacement		0	0	0	1	6	5	4	11
Marrow - Increased Granulopoiesis		5	7	7	9	7	5	4	3
Trachea	Number Examined:	66	66	66	65	65	65	64	66
Epithelial Hyperplasia		0	0	0	0	0	0	0	1
Inflammation/Ulceration		0	0	0	4	0	1	0	5
Inflammatory Exudate in Lumen		0	0	1	2	0	0	1	1
Larynx	Number Examined:	65	66	66	65	65	65	66	66
Foreign Material and Inflammatory Exudate		1	0	2	5	1	4	2	4
Mucosal Ulceration/Inflammation		0	1	0	6	0	2	0	5
Lungs + Bronchi	Number Examined:	66	66	66	66	66	65	66	66
Alveolar Haemorrhage		4	4	7	6	2	6	3	2
Bronchioloalveolar Hyperplasia		3	5	3	4	2	0	4	5
Bronchopneumonia		0	0	0	0	0	0	0	1
Inflammation in Bronchial Lumen		0	0	0	0	0	0	1	1
Peribronchiolar Inflammatory Cells		0	0	0	1	0	0	0	0
Skin	Number Examined:	66	66	66	66	66	66	66	66
Epidermal Hyperplasia/Hyperkeratosis		1	1	3	2	3	1	1	4
Epidermal Ulceration		4	3	4	9	7	3	1	4
Inflammation/Necrosis		4	3	6	10	6	4	3	4
Oedema		0	1	2	3	1	3	0	1
Scab(s)		3	4	4	7	5	2	2	3
Tail	Number Examined:	1	4	0	0	4	1	3	5
Epidermal Ulceration		0	0	0	0	0	0	0	1
Harderian Glands	Number Examined:	66	66	65	66	65	66	66	66
Acinar Hyperplasia		4	1	4	6	2	2	1	3
Acinar Necrosis/Atrophy/Basophilia with Inflammation		8	9	7	17	7	2	1	4
Lymphoid Aggregates		22	22	12	17	24	21	24	30
Optic nerves	Number Examined:	62	60	64	57	61	61	55	57
Gliosis		7	2	6	10	3	5	0	7
Seminal Vesicles	Number Examined:	66	66	66	66	-	-	-	-
Atrophy		0	0	0	1	-	-	-	-
Epithelial Hyperplasia		9	4	6	13	-	-	-	-
Inflammation/Fibrosis		2	7	2	2	-	-	-	-

Group	:	1	2	3	4
Compound	:	Control	VEC-162	VEC-162	VEC-162
Dose (mg/kg/day)	:	0	30	100	300

Tissue and Finding	Group/Sex:	1M	2M	3M	4M	1F	2F	3F	4F
	Number:	66	66	66	66	66	66	66	66
Epididymides	Number Examined:	66	66	66	66	-	-	-	-
Degenerate Spermatogenic Cells in Duct(s)		6	10	8	4	-	-	-	-
Lymphoid Infiltration		3	7	5	3	-	-	-	-
Spermatazoa Absent		6	14	9	8	-	-	-	-
Spermatocele Granuloma		1	5	0	1	-	-	-	-
Testes	Number Examined:	66	66	66	66	-	-	-	-
Seminiferous Tubular Atrophy		17	28	21	19	-	-	-	-
Seminiferous Tubular Dilatation		0	0	0	2	-	-	-	-
Sperm Stasis		10	18	13	8	-	-	-	-
Spermatocele Granuloma(ta)		0	0	1	0	-	-	-	-
Preputial glands	Number Examined:	66	63	66	66	-	-	-	-
Cystic Atrophy		36	44	54	57	-	-	-	-
Granulomatous Inflammation		4	9	10	4	-	-	-	-
Ovaries	Number Examined:	-	-	-	-	65	66	66	66
Absent Corpora Lutea		-	-	-	-	3	5	5	2
Haemorrhagic Cyst(s)		-	-	-	-	12	15	16	17
Sertoliform Tubular Hyperplasia		-	-	-	-	1	2	3	1
Uterus	Number Examined:	-	-	-	-	66	66	66	66
Cyst(s)		-	-	-	-	2	3	2	5
Luminal Haemorrhage		-	-	-	-	2	1	4	1
Uterine cervix	Number Examined:	-	-	-	-	66	64	65	66
Glandular Squamous Metaplasia		-	-	-	-	0	3	2	1

Toxicokinetics [Week 4 and Week 26]

TK was assessed in a satellite group of animals. The sampling schedule was provided by the sponsor (below). Satellite animals were discarded without necropsy following scheduled sampling.

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Week No	Group/Sex	Time of sampling (hours after dosing) 1	2	4	8	12	24
		Animal Number					
4	1M	67-69	70-72	73-75	76-78	79-81	82-84
	1F	487-489	490-492	493-495	496-498	499-501	502-504
	2M	172-174	175-177	178-180	181-183	184-186	187-189
	2F	592-594	595-597	598-600	601-603	604-606	607-609
	3M	277-279	280-282	283-285	286-288	289-291	292-294
	3F	697-699	700-702	703-705	706-708	709-711	712-714
	4M	382-384	385-387	388-390	391-393	394-396	397-399
	4F	802-804	805-807	808-810	811-813	814-816	817-819

Week No	Group/Sex	Time of sampling (hours after dosing) 1	2	4	8	12	24
		Animal Number					
26	1M	85-87	88-90	91-93	94-96	97-99	100-102
	1F	505-507	509, 510, 523	511-513	514-516	517-519	520-522
	2M	190-192	193-195	196-198	199-201	202-204	205-207
	2F	610-612	613-615	616-618	619-621	622-624	625-627
	3M	295-297	298-300	301-303	304-306	307, 308, 315	310, 313, 314
	3F	715, 716, 733	718-720	721-723	724-726	727-729	730, 732, 734
	4M	400-402	403-405	406-408	409-411	412-414	415-417
	4F	820-822	823-825	826-828	829-831	832-834	836, 837, 838

Some control samples were found to contain tasimelteon, and were confirmed upon re-examination. A total of three animals (out of 36) were affected, and plasma levels were low (4-20 ng/mL). Absorption was relatively rapid, and plasma exposures increased (generally less than or approximately dose-proportionately, with the exception of LD::MD in Week 4) with increasing dose. See the sponsor's summary PK table, next page. There were no clear, consistent sex differences in plasma exposure (HDF at Week 4 excluded). There was some evidence of accumulation at LD and at HD between Weeks 4 and 26 (see the sponsor's table, below). The terminal rate constants and corresponding half-lives could not be calculated for all groups; the sponsor believes that tasimelteon may show time-dependent kinetics.

Dose level (mg/kg/day)	Accumulation ratio Males	Females
30	2.2	1.8
100	1.1	0.64
300	1.8	2.0

TABLE 7

Pharmacokinetic parameters of VEC-162 during Weeks 4 and 26 of 104 weeks of daily oral (gavage) administration of VEC-162 to mice

Week 4

Males

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (hours^{-1})	t½ (hours)
30	1550	1	BLQ	2890	0.7651	0.9
100	3900	2	BLQ	20900	a	-
300	9690	2	3.50	48100	a	-

Females

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (hours^{-1})	t½ (hours)
30	1330	1	BLQ	3250	0.4688	1.5
100	8320	1	BLQ	25700	0.5032	1.4
300	8890	1	BLQ	31400	0.6254	1.1

Week 26

Males

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (hours^{-1})	t½ (hours)
30	2560	1	BLQ	6500	0.6358	1.1
100	4920	1	BLQ	22100	a	-
300	15700	1	BLQ	88300	0.4739	1.5

Females

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{24} (ng.h/mL)	k (hours^{-1})	t½ (hours)
30	2330	1	BLQ	5850	0.8790	0.8
100	4470	1	BLQ	16400	a	-
300	17400	1	BLQ	61400	0.5503	1.3

a Terminal rate constant could not be adequately estimated

Dosing Solution Analysis

The drug substance was generally stored refrigerated (2-8°C; although batch 800156790 was stored at ambient temperature, 21°C) and protected from light. All formulations were prepared weekly and stored refrigerated (2 to 8°C) and protected from light. The sponsor stated that homogeneity and stability were demonstrated in the rat carcinogenicity study (TAJ0001) at 7.5 and 100 mg/mL for 24 hr at ambient temperature (nominally 21°C) and for 15 days refrigerated (nominally 2-8°C). Formulations prepared for Weeks 1, 13, 26, 39, 52, 65, 78, 91 and 103 were analyzed for drug concentration. Four samples (nominally 1 mL accurately weighed) from all formulations were obtained, from which two samples were analyzed (the remaining two samples were retained frozen). The formulation concentrations were within ±7% of nominal, and individual results were within 3% of the mean.

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Interspecies Parent and Metabolite Comparison
For reference, the sponsor provided the following summary estimates of M11 exposure (see sponsor's Table 14 from the Nonclinical Toxicology Written Summary, following). The focus was solely on metabolite M11 (not a major human circulating metabolite) because rats do not produce M11 *in vivo*.

Table 14: Tasimelteon metabolite levels estimated from tasimelteon plasma levels measured at 26 weeks in the mouse carcinogenicity study (Study TAJ0002) using the metabolite/tasimelteon AUC ratios determined in Study TAJ0018

		AUC_{0-24} (ng.h/mL)		
Mouse		Tasimelteon	M11 ratio[a]	M11
100 mg/kg	M	22,100	0.0037	82
	F	16,400	0.0021	34
300 mg/kg	M	88,300	0.0037	327
	F	61,400	0.0027	166
Human[b] 20 mg QD		363		145

[a] Metabolite/tasimelteon AUC ratios from Study TAJ0018 at the same doses (corrected for molecular weights).
[b] Study VP-VEC-162-1110: An Open-Label, Single Sequence Study to Assess the Effect of Multiple Doses of Tasimelteon on the Cytochrome P450 3A4 and 2C8 Enzymes using Midazolam and Rosiglitazone as Substrates.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

As suggested by some effects in the toxicity studies, several effects were demonstrated in the reproductive toxicology studies. The fertility study in rats tested 0, 5, 50, and 500 mg/kg tasimelteon (BMS-214778) in males and females, and then females only (separately) after a reduction in fertility was observed. At HD, 3 HDF were euthanized moribund. Treatment with tasimelteon caused a clear reduction in body weight in males, but only a transient effect in females. Two HDM showed small male reproductive organs (i.e., testes and/or epididymides); however, matings with these males resulted in pregnancies within 1-2 days. Irregular estrus cycles were observed in MD and HD females. Although >90% of the animals mated during the first receptive period, increased infertile matings and a reduction in fertility parameters were observed at MD and HD. A re-evaluation was conducted pairing the treated males with untreated females; no effect on fertility was observed.

Study Title: BMS-214778: ORAL STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN RATS

Study no.:	Bristol-Myers Squibb Study No.99009 (b) (4) Reference No.TAJ0008/072447
Study report location:	EDR
Conducting laboratory and location:	(Live phase- all raw data, dated 1999) BMS, Pharmaceutical Research Institute, Department of Pathology, New Brunswick, NJ (Test article and final report, dated 2007) (b) (4) (b) (4)
Date of study initiation:	2/11/99
GLP compliance:	Yes (b) (4) & BMS)
QA statement:	Yes (b) (4) & BMS)
Drug, lot #, and % purity:	BMS-214778, NO31C-214778-01, 99.3%

Methods (see the sponsor's summary of the design, below)

Frequency of dosing: M: QD for 4 weeks before pairing until termination
F: QD for 2 weeks before pairing until D7 after mating

Dose volume: 3 mL/kg

Route of administration: PO, oral gavage

Formulation/Vehicle: Polyethylene glycol-400, PEG-400 Lot number M18621

Species/Strain: Crl:CD®(SD)IGS BR rats

(b) (4)

8-9 weeks of age at initiation
M: 292-331 g, F: 208-288 g

Amendments/Deviations to/from study protocol: **Amendment 1**: Fertility rates were decreased at MD and HD in treated female rats. The sponsor added a group of untreated females to be bred with the treated males, in order to determine "whether male or female reproductive function was affected by drug treatment."
Amendment 2: BMS decided to discontinue development of BMS-214778; a final report was not issued.
Amendment 3: Documents the change in "ownership" of the study and study report.

Group	Treatment	Dose (mg/kg/day)	No. of animals Male	No. of animals Female	Animal numbers@ Male	Animal numbers@ Female
1	Control	0	25	25	101-125	1-25
2	BMS-214778	5	25	25	126-150	26-50
3	BMS-214778	50	25	25	151-175	51-75
4	BMS-214778	500	25	25	176-200	76-100

Extension (Only Males Treated):

Group	Male Treatment	Dose (mg/kg/day)	No. of animals Male	No. of animals Female	Animal numbers@ Treated Male	Animal numbers@ Untreated Female
1	Control	0	25	25	101-125	201-225
2	BMS-214778	5	25	25	126-150	226-250
3	BMS-214778	50	25	25	151-175	251-275
4	BMS-214778	500	25	25	176-200	276-300

Observations and Results

Dosing Solution Analysis [1st and last weeks]

The concentrations of the tasimelteon (BMS-214778; batch N031C-214778-01) formulations were within ±5% of nominal.

Mortality [Twice daily]

The sponsor reported no drug-related deaths in males, LDF, and MDF.

The sponsor reported that three HDF were euthanized in moribund condition after 11 daily doses; in these animals, clinical signs of perioral, perinasal, and periocular substance, urine-stained and ungroomed coat, lethargy, and dehydrated skin were observed. Two of these animals also showed reduced, soft, and/or loose feces and/or fecal stained coat, fluid in the large intestines, and/or dark red discoloration of the liver. Necropsy did not identify a clear cause of death for any of these animals.

In addition to these animals, 1 conM and 1 LDM were found dead after 42 and 87 daily doses, respectively. One LDM was euthanatized due to excessive aggression following 25 daily doses. No drug-related changes were reported in any of these animals at necropsy.

Four males (2 LDM, 1 MDM, and 1 HDM) and two females (1 LDF and 1 HDF) were found dead or euthanized in moribund condition from intubation accidents (after 5 to 70 daily doses); one or more findings consistent with intubation injuries were noted at necropsy).

All other rats survived to scheduled necropsy.

Clinical Signs [Daily, prior to dosing and 1 hr post-dose]

In males, drug-related clinical signs occurred at all doses, consisting of dose-related increased incidences of perioral substance (red, brown, and/or clear), fecal-stained coat, and low incidences of periocular substance (red, brown and/or clear). Urine-stained and ungroomed coat and low incidences of soft feces were observed at MD and HD. Decreased motor activity and reduced feces were observed in one HDM.

In females, dose-related increased incidences of perinasal and perioral substance were observed. At HD, increased incidences of periocular substance, ungroomed coat, fecal- and/or urine-stained coat, and localized alopecia were observed. Low incidences of reduced feces, decreased motor activity, and dehydrated skin were also observed at HD.

Body Weight [M: Twice weekly, F: Twice weekly during treatment and post-mating D0, 4, 7, 10, 13, and 16]

Average body weights were dose-dependently decreased in males. At HD, body weight gain was reduced in the first five days of treatment (~5%), compared to control. At the first pairing (D29), average body weights of treated males were 5 - 11% lower than

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those of controls. At D92, treated males weighed on average 5 - 16% less than controls. See the sponsor's figure, below.

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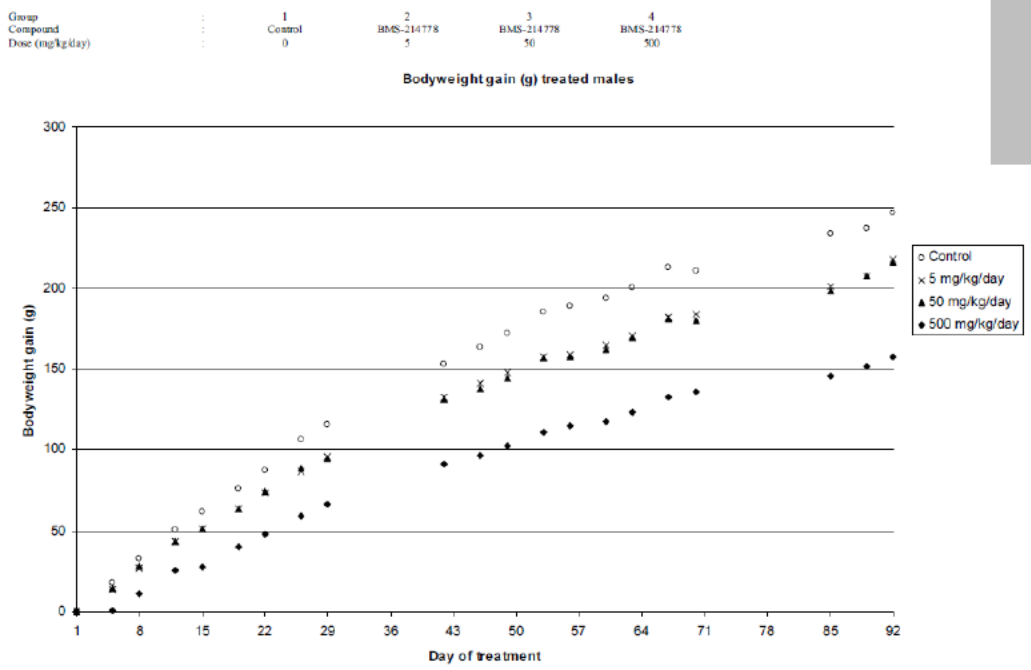
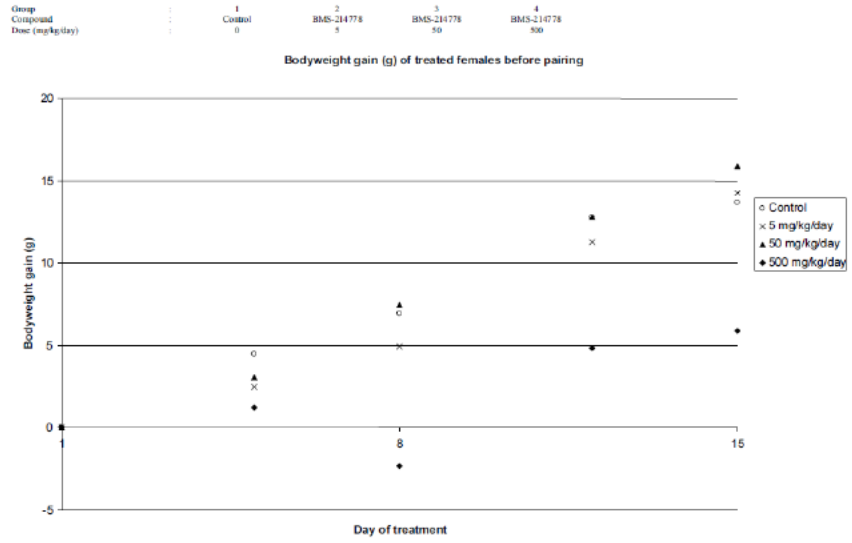


There was no clear effect on body weight in females at LD and MD. HD females showed average body weight loss during the first week of treatment, and average body weight continued to be slightly reduced (~2%, compared to controls) through pairing. During gestation, average weight gain remained reduced compared to control through the treatment period (GD7, up to 4%), and then showed recovery from GD7 through GD16 (< 2% reduction compared to controls).



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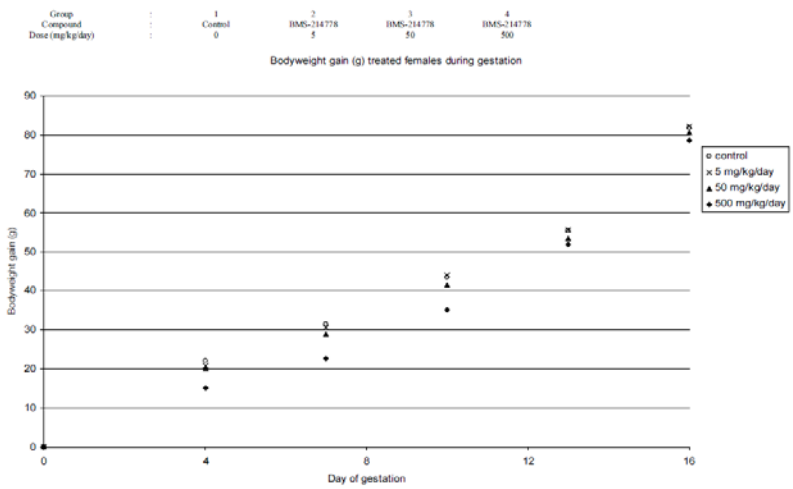

Food Consumption [Weekly during treatment, for F: twice weekly GD]
There was no clear effect on food consumption at LD and MD. Food consumption was reduced (84 - 90% of control values) in HDM during the four weeks before first pairing, and remained lower than controls throughout the study. In HDF, food consumption was reduced throughout treatment (80-87% of controls, before pairing to GD7), then was similar to controls after treatment ceased.

Toxicokinetics [N/A]

Necropsy [M: D92 of treatment, F: GD16]
The sponsor reported no gross lesions in animals not dying prior to scheduled termination. However, 2 HDM (animals 4M0180 and 4M0192) were observed to show small right testes and small and/or short right epididymides (mating with both of these males resulting in pregnancies within 1-2 days).

Estrus cycles
Before treatment, females showed estrus cycles of 4 or (rarely) 5 days duration. There was no clear effect of drug at LD. In MDF and HDF, drug was associated with an increased incidence of females showing irregular cycles. See the sponsor's summary data, below.

GROUP VALUES DURING TREATMENT

Group	Number of animals		Regular cycles 4 day	4/5 day	5 day	Irregular cycle λ	Extended estrus ϕ	Acyclic ψ
1	25	n	18	5	0	1	0	1
		(%)	(72)	(20)		(4)		(4)
2	24	n	18	2	0	2	0	2
		(%)	(75)	(8)		(8)		(8)
3**	25	n	10	4	0	8	2	1
		(%)	(40)	(16)		(32)	(8)	(4)
4**	21	n	3	8	0	6	3	1
		(%)	(14)	(38)		(29)	(14)	(5)

λ At least one cycle of two, three or six to ten days
ϕ At least four consecutive days of estrus
ψ At least ten days without estrus
Statistical analysis - exact one-tailed (upper-tail) Linear-by-linear test
Stars next to a group number indicate statistical significance for the test including all group numbers up to and including the one they are presented against: ** p<0.01.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Animals were paired one-to-one for a period of up to 3 weeks. The day on which evidence of mating occurred was designated GD0, and the animals were separated. In the extension, untreated females were paired with the surviving males for a maximum of 14 days.

At least 92% of animals mated during the first estrous period (see sponsor's Text Table 1). Infertile pairings were increased at MD and HD; the sponsor indicated that most of these resulted from mating on the first night at an infertile stage of the female's cycle. However, there was a slight reduction in fertility at MD and HD (see the sponsor's summary table, below).

Text Table 1: Individual mating information for infertile pairings, treated females/treated males

Group	Treated Female	Treated male	Final cycle length (days)	PCI (days)	Copulation plugs seen (Y/N)	Sperm detected (Y/N)	Comment
1	8	108	4	2	Y	Y	Normal mating
2	30	130	3	1	N	Y	Early mating
3	52	152	5	1	Y	Y	Late mating
3	55	155	2	1	N	Y	Early mating
3	59	159	5	1	N	Y	Late mating
3	72	172	3	2	Y	Y	Early mating
4	81	181	3	1	N	Y	Early mating
4	88	188	12	3	Y	Y	Mated after acyclic period
4	90	190	8	2	N	Y	Mated after extended estrus

PCI - pre-coital interval

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TABLE 14

BMS-214778: ORAL STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN RATS

SUMMARY OF CESAREAN-SECTIONING OBSERVATIONS IN TREATED FEMALE RATS MATED WITH TREATED MALE RATS

Group		1	2	3	4
Compound		Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)		0	5	50	500
GROUP		1	2	3	4
RATS COHABITED	N	25	24	25	21
RATS MATED[a]	N	25	24	25	21
RATS PREGNANT[b]	N(%)	24(96.0)	23(95.8)	21(84.0)	18(85.7)
PREGNANT RATS SURVIVING TO DAY 16 CESAREAN-SECTIONING	N	24	23	21	18
CORPORA LUTEA	MEAN	15.8	16.7	16.9	15.8
	SD	2.2	2.0	2.2	2.1
IMPLANTATIONS	MEAN	15.5	16.0	15.7	15.2
	SD	2.2	2.3	3.6	2.2
PREIMPLANTATION LOSS[c]	MEAN%	2.1	4.2	8.0	3.8
	SD	3.7	6.5	16.8	7.1
POSTIMPLANTATION LOSS[d]	MEAN%	4.3	7.3	3.9	5.7
	SD	6.3	10.2	4.8	10.4
LITTER SIZE (LIVE FETUSES)	N	355	339	316	259
	MEAN	14.8	14.7	15.0	14.4
	SD	2.3	2.2	3.5	2.8
EARLY RESORPTIONS	N	16	29	13	14
	MEAN	0.7	1.3	0.6	0.8
	SD	1.0	1.8	0.7	1.2
DAMS WITH ANY RESORPTIONS	N(%)	10(41.7)	11(47.8)	10(47.6)	10(55.6)
DAMS WITH NO VIABLE CONCEPTUSES	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Statistical Analysis: Analysis of Variance with Dunnett's procedure used for continuous data.
Kruskal-Wallis with Dunn's procedure used for enumeration data.
Fisher' Exact test used for proportion data.
No significant intergroup differences.

a - Restricted to rats with confirmed dates of mating.
b - Restricted to pregnant rats with confirmed dates of mating.
c - Preimplantation loss calculated as: [(Corpora lutea - implantations)/corpora lutea] x 100.
d - Postimplantation loss calculated as: [(Implantations - live fetuses)/implantations] x 100.

Due to the slight reduction in fertility at MD and HD, the males were re-assessed after 10 weeks of treatment by pairing with untreated females (see sponsor's summary Tables 16 and 20, below). There was no evidence that treatment of the male rats had any adverse effect upon litter parameters when mated with untreated females.

TABLE 16

BMS-214778: ORAL STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN RATS

SUMMARY OF MATING PERFORMANCE AND FERTILITY - GROUP VALUES - TREATED MALE RATS WITH UNTREATED FEMALE RATS

Group	1	2	3	4
Compound	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	0	5	50	500

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	24	24	22	100	92	92
2M	22	22	22	100	100	100
3M	24	24	23	100	96	96
4M	24	22	21	92	95	88

Statistical analysis - exact one-tailed (lower-tail) Cochran-Armitage test: No significant intergroup differences.

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TABLE 20

BMS-214778: ORAL STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN RATS

SUMMARY OF CESAREAN-SECTIONING OBSERVATIONS IN UNTREATED FEMALE RATS MATED WITH TREATED MALE RATS

Group	:	1	2	3	4
Compound	:	Control	BMS-214778	BMS-214778	BMS-214778
Dose (mg/kg/day)	:	0	5	50	500
GROUP		1	2	3	4
RATS COHABITED	N	24	22	24	24
RATS MATED[a]	N	24	22	24	22
RATS PREGNANT[b]	N(%)	22(91.7)	22(100.0)	23(95.8)	21(95.5)
PREGNANT RATS SURVIVING TO DAY 16 CESAREAN-SECTIONING	N	22	22	23	21
CORPORA LUTEA	MEAN	16.7	16.6	17.0	18.0
	SD	2.1	1.4	1.5	1.9
IMPLANTATIONS	MEAN	16.2	16.1	16.4	17.8
	SD	2.3	2.0	2.3	2.0
PREIMPLANTATION LOSS[c]	MEAN%	3.4	3.4	3.5	1.1
	SD	6.0	8.7	9.0	3.0
POSTIMPLANTATION LOSS[d]	MEAN%	2.4	6.7*	3.9	1.8
	SD	3.9	7.1	6.3	4.9
LITTER SIZE (LIVE FETUSES)	N	347	330	362	367
	MEAN	15.8	15.0	15.7	17.5*
	SD	2.2	2.2	2.3	2.3
EARLY RESORPTIONS	N	9	24	15	6
	MEAN	0.4	1.1	0.7	0.3
	SD	0.7	1.2	1.1	0.7
DAMS WITH ANY RESORPTIONS	N(%)	7(31.8)	13(59.1)	9(39.1)	4(19.0)
DAMS WITH NO VIABLE CONCEPTUSES	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Statistical Analysis: Analysis of Variance with Dunnett's procedure used for continuous data.
Kruskal-Wallis with Dunn's procedure used for enumeration data.
Fisher' Exact test used for proportion data.

* Significantly different from control at P ≤ 0.05.

a - Restricted to rats with confirmed dates of mating.
b - Restricted to pregnant rats with confirmed dates of mating.
c - Preimplantation loss calculated as: [(Corpora lutea - implantations)/corpora lutea] x 100.
d - Postimplantation loss calculated as: [(Implantations - live fetuses)/implantations] x 100.

9.2 Embryonic Fetal Development

The EFD study in rats tested 0, 5, 50, and 500 mg/kg BMS-214778. Maternal toxicity (morbidity, decreased body weight and food consumption) was observed at HD. Some embryofetal toxicity (i.e., slightly delayed development at MD and HD and slightly decreased fetal weight at HD) was observed. Delayed development was observed as an increase in skeletal variations (i.e., incomplete ossification).

Study Title: ORAL STUDY OF ENBRYO-FETAL DEVELOPMENT IN RATS

Study no.:	99005
Study report location:	EDR
Conducting laboratory and location:	Bristol-Myers Squibb, Pharmaceutical Research Institute, New Brunswick, NJ
Date of study initiation:	Not given, protocol dated 1/28/99
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-214778, batch N031C-214778-01, 99.8%

Key Findings:

- Maternal toxicity (mortality, decreased body weight and food consumption) at 500 mg/kg
- Some embryofetal toxicity, including slightly delayed development at 50 and 500 mg/kg and slightly decreased fetal weight at 500 mg/kg

Methods

Doses:	0, 5, 50, & 500 mg/kg
Frequency of dosing:	QD, GD6-15
Dose volume:	3 ml/kg
Route of administration:	PO, oral gavage
Formulation/Vehicle:	PEG 400
Species/Strain:	Crl:CD®(SD)IGS BR Sprague Dawley F rats 203-225 g, 8-10 weeks of age (b) (4)
Number/Sex/Group:	22 F
Study design:	Dose selection based on BMS-214778: Ten-Day Oral Range-Finding Study in Pregnant Rats (Study No. 98046). Evaluations on GD20. Dam evaluations: survival, clinical observations, body weight, food consumption & uterine contents Fetal evaluations: viability, gender, body weight, and gross external, visceral, and/or skeletal alterations.
Important Deviations from study protocol:	Tissues (i.e., uterus, ovaries and uterine contents) from the one HD dam sacrificed moribund on GD11 were discarded.

Observations and Results

Dosing Solution Analysis

Solutions were prepared as needed and used within 7 days; stability had been previously shown. The solutions used were within ±10 % of nominal.

Mortality [2x daily]
There were no drug-related LD and MD maternal deaths. One drug-related death occurred at HD.

The HD dam was euthanized in moribund condition on GD11. This rat had lost weight beginning on GD8 and had reduced food consumption starting GD9. Clinical signs included perioral, perinasal and/or periocular substance (clear, red or brown), reduced and/or soft feces, urine-stained and/or ungroomed coat on GD8-11. Necropsy did not reveal gross lesions. There were 13 embryos present *in utero*, which were reported as normal for gestational age.

Clinical Signs [up to 2x daily; pre-dose and 1 hr post-dose]
There were no drug-related signs at LD or MD. At HD, perioral and perinasal substance (12/22; clear, red or brown), reduced feces (10/22), localized alopecia (6/22; hindlimbs, dorsal surface or ventral surface), urine- and/or fecal-stained coat (4/22), and decreased motor activity (3/22; including ataxia in 1; over 1-2 days) were observed.

Body Weight [daily]
The numbers of dams in control, LD, MD, and HD groups were 15, 18, 20, and 22/21; non-pregnant animals were not included. Also, two dams were excluded from the control mean due to having litters with a single fetus. No changes occurred at LD or MD. Significant decreases in body weight (3-9%) and body weight gains (including losses) occurred during the dosing period (GD6-15) in HD dams. Statistical significance was reached by GD9, and remained through GD16. Increased body weight gains were noted at HD over GD16-20.

Food Consumption [daily]
No changes occurred at LD. Food consumption was transiently slightly decreased at MD on GD6 to GD7. Significant decreases in food consumption occurred at HD during the dosing period (GD6-16; averaging ~25%), with increases noted during GD16-20.

Toxicokinetics [N/A]

Necropsy [GD20]
All dams were reported as normal.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss)
No drug-related changes in maternal or litter parameters at cesarean-sectioning were observed. Numbers of corpora lutea and implantations, litter sizes, numbers of live and·dead fetuses, resorptions, and fetal sex ratios were comparable among groups. Fetal weight was very slightly reduced (~3% [nss]) at HD. See the sponsor's summary tables, provided below.

Reference ID: 3399188

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GROUP		1	2	3	4
TREATMENT		Control	BMS-214778	BMS-214778	BMS-214778
DAILY DOSE (mg/kg/day)		0	5	50	500
RATS EVALUATED	N	22	22	22	22
RATS PREGNANT	N(%)	17(77.3)	18(81.8)	20(90.9)	22(100.0)
PREGNANT RATS SURVIVING TO DAY 20 CESAREAN-SECTIONING	N	15[a]	18	20	21
CORPORA LUTEA	MEAN	13.5	13.0	13.5	13.3
	SD	1.5	1.8	1.9	2.2
IMPLANTATIONS	MEAN	12.2	12.2	12.4	11.9
	SD	2.5	1.4	2.1	2.7
PREIMPLANTATION LOSS[b]	MEAN%	9.8	6.0	7.9	11.9
	SD	14.6	5.9	10.8	13.7
POSTIMPLANTATION LOSS[c]	MEAN%	2.9	4.8	4.0	2.3
	SD	5.8	4.5	6.9	5.0
LITTER SIZE (LIVE + DEAD)	MEAN	11.8	11.6	11.9	11.6
	SD	2.3	1.6	2.1	2.8
LIVE FETUSES	MEAN	11.8	11.6	11.9	11.6
	SD	2.3	1.6	2.1	2.8
DEAD FETUSES	N	0	0	0	0
RESORPTIONS (EARLY + LATE)	N	6	10	10	5
	MEAN	0.4	0.6	0.5	0.2
	SD	0.8	0.5	0.9	0.4
EARLY RESORPTIONS	N	6	10	10	5
	MEAN	0.4	0.6	0.5	0.2
	SD	0.8	0.5	0.9	0.4
LATE RESORPTIONS	N	0	0	0	0
DAMS WITH ANY RESORPTIONS	N(%)	4(26.7)	10(55.6)	6(30.0)	5(23.8)
DAMS WITH NO VIABLE CONCEPTUSES	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Statistical Analysis: Analysis of Variance with Dunnett's procedure used for continuous data.
Kruskal-Wallis test with Dunn's procedure used for enumeration data.
Fisher's exact test used for proportion data.

a - Excludes dams 1F0011 and 1F0021 which had single conceptus litters (the conceptus in litter 1F0021 was an early resorption; the conceptus in litter 1F0011 was a live fetus).
b - Preimplantation loss calculated as: [(Corpora Lutea - implantations)/corpora lutea] x 100.
c - Postimplantation loss calculated as: [(Dead + resorbed conceptuses)/implantations] x 100.

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GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-214778 5	3 BMS-214778 50	4 BMS-214778 500
LITTERS WITH ONE OR MORE LIVE FETUSES ON DAY 20 OF GESTATION	N	15[a]	18	20	21
LIVE FETUSES	N	177	209	237	244
	MEAN	11.8	11.6	11.9	11.6
	SD	2.3	1.6	2.1	2.8
LIVE MALE FETUSES	N	83	104	117	109
% LIVE MALE FETUSES PER LITTER	MEAN%	45.2	49.9	50.0	42.6
	SD	18.3	15.2	10.7	16.4
MEAN FETAL BODY WEIGHT (Grams) PER LITTER	MEAN	3.91	3.93	3.92	3.80
	SD	0.21	0.26	0.20	0.20
MALE FETUSES	MEAN	3.97[b]	4.03	4.00	3.90[c]
	SD	0.23	0.27	0.23	0.20
FEMALE FETUSES	MEAN	3.84	3.84	3.83	3.72
	SD	0.22	0.24	0.22	0.20
% DEAD OR RESORBED CONCEPTUSES PER LITTER	MEAN%	2.9	4.8	4.0	2.3
	SD	5.8	4.5	6.9	5.0

NOTE: All values were calculated on the basis of live fetuses in each group.

Statistical Analysis: Analysis of Variance with Dunnett's procedure used for continuous data.
Kruskal-Wallis test with Dunn's procedure used for enumeration data.

a - Excludes litter 1F0011 which consisted of one male fetus.
b - N=14; litter 1F0020 had no male fetuses
c - N=20; litter 4F0078 had no male fetuses.

Offspring (Malformations, Variations, etc.)

All fetuses were evaluated for gross external alterations; half were examined for visceral alterations and half were examined for skeletal alterations (KOH-alizarin red-S technique). Notably, no malformations were reported in any group, which is unusual. There were no clearly drug-related effects. See the sponsor's summary table of alterations, below. A slight increase in skeletal variations was observed but there was not a clear dose relationship; incomplete ossification suggested a very slight delay in development (e.g., dumb-bell shaped thoracic vertebrae) at MD and HD.

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GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-214778 5	3 BMS-214778 50	4 BMS-214778 500
FETUSES EVALUATED	N	178	209	237	244
Live Fetuses	N	178	209	237	244
Dead Fetuses	N	0	0	0	0
LITTERS EVALUATED	N	16	18	20	21
ALTERATIONS					
(Malformations + Variations)					
Fetuses with Any Alterations	N(%)	15(8.4)	16(7.7)	28(11.8)	14(5.7)
Litters with Fetuses with Any Alterations	N(%)	7(43.8)	11(61.1)	14(70.0)	8(38.1)
Percent Fetuses per Litter with Alterations	MEAN	7.8	7.5	11.4	5.4
	SD	10.0	6.9	10.0	8.6
VARIATIONS[a]					
Fetuses with Any Variations	N(%)	15(8.4)	16(7.7)	28(11.8)	14(5.7)
Litters with Fetuses with Any Variations	N(%)	7(43.8)	11(61.1)	14(70.0)	8(38.1)
Percent Fetuses per Litter with Variations	MEAN	7.8	7.5	11.4	5.4
	SD	10.0	6.9	10.0	8.6
MALFORMATIONS[b]					
Fetuses with Any Malformations	N(%)	0	0	0	0
Litters with Fetuses with Any Malformations	N(%)	0	0	0	0
Percent Fetuses per Litter with Malformations	MEAN	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0

NOTE: All values were calculated on the basis of live fetuses in each group.

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data. Fisher's exact test was used for proportion data.

a - Common observations in this species and strain and reversible delays or accelerations in development.
b - Irreversible changes occurring at low incidence in this species and strain.

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Excerpts from the sponsor's summary table for skeletal observations:

GROUP TREATMENT DAILY DOSE (mg/kg)		1 Control 0	2 BMS-214778 5	3 BMS-214778 50	4 BMS-214778 500
FETUSES EVALUATED	N	90	107	118	123
Live Fetuses	N	90	107	118	123
Dead Fetuses	N	0	0	0	0
LITTERS EVALUATED	N	16	18	20	21
STERNEBRAE: Dumbbell-shaped (V)					
Fetal Incidence	N(%)	0	1(0.9)	4(3.4)	4(3.3)
Litter Incidence	N(%)	0	1(5.6)	3(15.0)	2(9.5)
STERNEBRAE: Cleaved (V)					
Fetal Incidence	N(%)	0	0	0	1(0.8)
Litter Incidence	N(%)	0	0	0	1(4.8)
VERTEBRAE: Thoracic, centra, bifid (V)					
Fetal Incidence	N(%)	0	0	3(2.5)	1(0.8)
Litter Incidence	N(%)	0	0	3(15.0)	1(4.8)
VERTEBRAE: Thoracic, centra, dumbbell-shaped (V)					
Fetal Incidence	N(%)	4(4.4)	3(2.8)	8(6.8)	6(4.9)
Litter Incidence	N(%)	3(18.8)	3(16.7)	6(30.0)	3(14.3)
VERTEBRAE: Lumbar, centra, bifid (V)					
Fetal Incidence	N(%)	0	0	0	1(0.8)
Litter Incidence	N(%)	0	0	0	1(4.8)
VERTEBRAE: Lumbar, centra, dumbbell-shaped (V)					
Fetal Incidence	N(%)	3(3.3)	0	0	0
Litter Incidence	N(%)	2(12.5)	0	0	0

NOTE: All values were calculated on the basis of live fetuses in each group.
(V) = Variation

The EFD study in rabbits tested 0, 5, 30, and 200 mg/kg. Maternal toxicity (i.e., clinical signs, reductions in body weight and food consumption) was observed at HD. Black ovarian cysts were observed in treated does, and there was an increase in abortions at HD. Embryofetal effects at HD included increased post-implantation losses (late resorption in MD and HD) and slightly reduced fetal weights, with some suggestion that male fetuses were slightly more affected. Also at HD, an increase in the incidence of incompletely ossified pubes (indicating a developmental delay) and a slight increase in total variations and malformations were observed (generally not due to any specific alteration). The appearance of a general slight increase in variations and malformations may have resulted from the large number of abortions at HD (resulting in a reduced number of fetuses).

Study Title: ORAL STUDY OF EMBRYO-FETAL DEVELOPMENT IN RABBITS

Study no.:	99001
Study report location:	EDR
Conducting laboratory and location:	Bristol-Myers Squibb, Pharmaceutical Research Institute, New Brunswick, NJ
Date of study initiation:	Not given, protocol dated 1/15/99
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-214778, (Batch No. N031C-214778-01, 99.3%

Key Findings:

- Maternal toxicity at 200 mg/kg (clinical signs, decreased bodyweight, & food consumption)
- Embryo-fetal toxicity (aborted litters, slightly decreased fetal weight, some evidence for increases in malformation/variation rates, and slightly delayed ossification, esp. pelvis) at 200 mg/kg.

Methods

Doses:	0, 5, 30, & 200 mg/kg
Frequency of dosing:	QD, GD 7- 19
Dose volume:	1 ml/kg
Route of administration:	PO, oral gavage
Formulation/Vehicle:	polyethylene glycol-400 (PEG-400)
Species/Strain:	Hra:(NZW)SPF rabbits (b) (4) nulliparous, timed-mated, 5-6 mo of age, 2.85 to 4.24 kg
Number/Sex/Group:	20 F/group
Study design:	Dose selection based on BMS-214778: Thirteen-Day Oral Range-Finding Study in Pregnant Rabbits (Study No. 98052). Evaluations on GD29: Dam evaluations: survival, clinical observations, body weight, food consumption & uterine contents Fetal evaluations: viability, gender, body weight, and gross external, visceral, and/or skeletal alterations.

Observations and Results

Dosing Solution Analysis

Solutions of tasimelteon (BMS-214778) in 100% PEG-400 were prepared as needed and used within 5 (200 mg/ml) or 7 days (5 and 30 mg/ml). The formulations were within ±10% of nominal concentration.

Reference ID: 3399188

Mortality [2x/daily]
There were no drug-related deaths during the study.

Two LD does did not survive to scheduled cesarean-sectioning on GD29. One (#31) was euthanatized in moribund condition on GD14 as the result of an intubation accident (verified by necropsy); this animal had 10 embryos present *in utero*, all of which appeared normal for their developmental age. The second LD doe (#30) was euthanatized in moribund condition on GD21 as the result of persistent bodyweight losses and anorexia; at necropsy, numerous gross lesions of the stomach, liver, gallbladder, intestines, kidneys, and ovaries were noted. The uterus contained 7 early resorptions as well as 5 fetuses described as "unremarkable." No cause of morbidity was determined for this doe.

Additionally, a number of does were euthanized following abortions. Increased incidence of abortion was observed at HD, although abortion of 1 or more fetuses occurred in all groups (see sponsor's Table 1, below) on GD19-GD29 prior to scheduled cesarean delivery. Necropsy observations included black and/or hardened stomach contents, ingested conceptuses and placentae, fluid-filled intestine(s), hardened gallbladder, thinned and/or pitted stomach lining, mottled liver, and ovarian cyst(s). All fetuses in the aborted litters were reported as "normal for their developmental age."

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STUDY NO. 99001

TABLE 1

BMS-214778: ORAL STUDY OF EMBRYO-FETAL DEVELOPMENT IN RABBITS

SUMMARY OF MORTALITY AND CLINICAL OBSERVATIONS

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-214778 5	3 BMS-214778 30	4 BMS-214778 200
RABBITS ASSIGNED TO STUDY	N	20	20	20	20
MORTALITY (Total):	N	2	4	1	5
Found Dead	N	0	0	0	0
Moribund-Euthanatized	N	0	2	0	0
Aborted	N	2	2	1	5
CLINICAL OBSERVATIONS:					
RABBITS EVALUATED[a]	N	20	20	20	20
Feces, soft	N	3(2)	8(3)	4(1)	13(5)
Feces, reduced and/or absent	N	5(2)	10(4)	9(1)	14(5)
Perivaginal substance, red	N	1(1)	2(2)	1(1)	5(5)
Red substance and/or tissue in cage pan	N	1(1)	2(2)	1(1)	5(5)
Coat, fecal-stained	N	14(2)	14(3)	11(1)	16(5)
Perioral substance, yellow, red and/or brown	N	0	2(2)	0	0
Perinasal substance, red	N	0	0	0	1(1)
Wheezing, labored breathing, and/or rales	N	0	2(2)	0	0
Localized alopecia, forelimb(s), hindlimb(s), tail, ventral surface and/or dorsal surface	N	0	4(2)	2	2(1)
Localized skin lesion, hindpaw(s), and/or ventral surface	N	0	1	0	1(1)
Decreased motor activity	N	0	1(1)	0	0
Urine, none	N	0	1(1)	0	1
Coat, urine-stained	N	3(2)	2	3(1)	2

() = Number of rabbits with the observation which died or were euthanatized prior to scheduled cesarean sectioning.

a - Values cited refer to the number of animals with the observation regardless of pregnancy status.

Clinical Signs [1-2x daily, predose & 1 hr post-dose]

Drug-related clinical signs at HD included increased incidences of soft, reduced, and absent feces, and observations associated with abortion (red peri-vaginal substance, red substance and/or tissue in cage pan). See sponsor's summary Table 1, above, for details.

Body Weight [Daily, GD7-29]
Average body weight loss was observed at HD (maximum of 2%); the GD7 average body weight was not regained until GD13. Decreased maternal body weights (5%) and body weight gain were observed at HD throughout the dosing period and remained at GD29. No clear drug-related maternal body weight differences were observed at LD or MD.

Food Consumption [Daily, GD7-29]
The sponsor reported decreased food consumption at HD during the dosing period. Decreased food consumption was observed in a few individuals, with the greatest number observed at HD (10 HD, 3 MD, 4 LD, and 1 control). Animals with continually very low food consumption often, but not always (1 LD, 3 MD, and 2 HD), aborted.

Necropsy [GD29]
No drug-related findings were reported in does surviving to GD29. Pale kidneys were observed in one MD doe. Small ovaries were observed in 2 LD does and 1 MD doe. Black ovarian cysts were noted in 2 LD (early mortalities), 1 MD, and 2 HD (early mortalities) does. Observations in other organs (e.g., stomach, intestines, liver, gall bladder, and kidneys) were only observed in early mortalities (see **Mortality** for details).

Cesarean Section Data
Intact gravid uteri (including ovaries) were weighed. Corpora lutea and implantation sites were counted and the placement of implantation sites, early and late resorptions, and live and dead fetuses were noted. Each placenta was examined for grossly for alterations.

The sponsor reported minimal drug-related decreases in fetal body weight at HD (≤ 5%; see sponsor's Table 7, below); no other drug-related findings were reported for maternal or litter parameters. However, post-implantation losses (due to resorptions) were increased in treated animals (although this was not dose-related). The increase appeared related to early resorptions at LD, but both early and late resorptions were increased (compared to controls) at MD and HD. See the sponsor's summary Table 6, below. Additionally, the sponsor excluded data from the following does in the summary data presented: 1 LD doe with complete post-implantation loss (8 early resorptions), 1 LD doe with a single implantation leading to a live birth, and 1 MD doe with complete post-implantation loss (2 implantations, 2 early resorptions). Male fetuses were very slightly more affected than females (see % live male fetuses per litter and mean fetal body weight, male).

Reference ID: 3399188

STUDY NO. 99001

TABLE 6

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BMS-214778: ORAL STUDY OF EMBRYO-FETAL DEVELOPMENT IN RABBITS

SUMMARY OF CESAREAN-SECTIONING OBSERVATIONS

GROUP		1	2	3	4
TREATMENT		Control	BMS-214778	BMS-214778	BMS-214778
DAILY DOSE (mg/kg/day)		0	5	30	200
RABBITS EVALUATED	N	20	20	20	20
RABBITS PREGNANT	N(%)	20(100.0)	20(100.0)	20(100.0)	20(100.0)
PREGNANT RABBITS SURVIVING TO DAY 29 CESAREAN-SECTIONING	N	18	14[c]	18	15
CORPORA LUTEA	MEAN	9.3	9.6	9.4	9.2
	SD	1.4	1.5	1.7	2.0
IMPLANTATIONS	MEAN	8.2	8.9	9.1	8.9
	SD	2.0	1.6	1.8	2.1
PREIMPLANTATION LOSS[a]	MEAN%	11.3	7.4	4.3	3.7
	SD	19.0	9.5	5.8	5.6
POSTIMPLANTATION LOSS[b]	MEAN%	0.6	8.1	4.4	2.7
	SD	2.4	17.8	7.9	4.8
LITTER SIZE (LIVE + DEAD)	MEAN	8.2	8.1	8.7	8.6
	SD	2.0	2.2	2.1	1.9
LIVE FETUSES	MEAN	8.2	8.1	8.7	8.6
	SD	2.0	2.2	2.1	1.9
DEAD FETUSES	N	0	0	0	0
RESORPTIONS (EARLY + LATE)	N	1	10	6	4
	MEAN	0.1	0.7	0.3	0.3
	SD	0.2	1.5	0.6	0.5
EARLY RESORPTIONS	N	1	10	3	3
	MEAN	0.1	0.7	0.2	0.2
	SD	0.2	1.5	0.4	0.4
LATE RESORPTIONS	N	0	0	3	1
DOES WITH ANY RESORPTIONS	N(%)	1(5.6)	4(28.6)	5(27.8)	4(26.7)
DOES WITH NO VIABLE CONCEPTUSES	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE CONCEPTUSES	N(%)	18(100.0)	14(100.0)	18(100.0)	15(100.0)

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data.
Kruskal-Wallis with Dunn's procedure used for enumeration data.
Fisher's Exact test was used for proportion data.

a - Preimplantation loss calculated as: [(Corpora Lutea - implantations)/corpora lutea] x 100.
b - Postimplantation loss calculated as: [(Dead + resorbed conceptuses)/implantations] x 100.
c - Excludes doe 2F0036 that had a single-conceptus litter consisting of one live fetus.

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TABLE 7

BMS-214778: ORAL STUDY OF EMBRYO-FETAL DEVELOPMENT IN RABBITS

SUMMARY OF FETAL OBSERVATIONS IN CESAREAN-DELIVERED LITTERS

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-214778 5	3 BMS-214778 30	4 BMS-214778 200
LITTERS WITH ONE OR MORE LIVE FETUSES ON DAY 29 OF GESTATION	N	18	14[a]	18	15
LIVE FETUSES	N	147	114	157	129
	MEAN	8.2	8.1	8.7	8.6
	SD	2.0	2.2	2.1	1.9
LIVE MALE FETUSES	N	73	46	76	62
% LIVE MALE FETUSES PER LITTER	MEAN%	51.3	40.3	46.8	47.1
	SD	19.2	16.7	21.6	18.4
MEAN FETAL BODY WEIGHT (Grams) PER LITTER	MEAN	40.95	40.33	40.15	38.11
	SD	5.45	5.16	4.58	6.86
MALE FETUSES	MEAN	41.65	41.93	40.65[b]	39.49[c]
	SD	5.68	6.91	4.25	6.61
FEMALE FETUSES	MEAN	39.55[d]	39.24	39.77	38.10
	SD	4.49	4.79	4.84	6.71
% DEAD OR RESORBED CONCEPTUSES PER LITTER	MEAN%	0.6	8.1	4.4	2.7
	SD	2.4	17.8	7.9	4.8

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data. Kruskal-Wallis with Dunn's procedure used for enumeration data.

a - Excludes doe 2F0036 that had single-conceptus litter consisting of one live fetus.
b - N=17; excludes litter 3F0045 which had no male fetuses.
c - N=14; excludes litter 4F0076 which had no male fetuses.
d - N=17; excludes litter 1F0014 which had no female fetuses.

Offspring

All fetuses were weighed and examined for gross external, visceral (fresh dissection) and skeletal (stained using KOH-alizarin red-S technique) alterations.

The only reported drug-related change was a slight increase in the incidence of fetuses with incompletely ossified pubes at HD (see sponsor's Table 11, below). The sponsor indicated that this delayed ossification was concomitant with reduced fetal bodyweight. Additionally, a slight increase variations was noted overall at HD. Externally, arched palate occurred in a HD fetus born to doe #61, and a number of skeletal variations occurred at HD (see sponsor's Table 11).

However, in addition to the increased incidence of variations, there was a slight increase in malformations observed at HD (and also LD; see sponsor's Table 8). Single

HD fetuses showed gross malformations or variations (see sponsor's Table 9). Spina bifida occurred in a fetus born to doe #69. Malrotated paws occurred in doe #75's litter, and discolored/misshapen hindlimbs and paws occurred in doe #76's (this was coded as a variation rather than a malformation).

No clear drug-related visceral alterations or differences in ossifications sites were observed.

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TABLE 8

BMS-214778: ORAL STUDY OF EMBRYO-FETAL DEVELOPMENT IN RABBITS

SUMMARY OF FETAL ALTERATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-214778 5	3 BMS-214778 30	4 BMS-214778 200
FETUSES EVALUATED	N	147	115	157	129
Live Fetuses	N	147	115	157	129
Dead Fetuses	N	0	0	0	0
LITTERS EVALUATED	N	18	15	18	15
ALTERATIONS (Malformations + Variations)					
Fetuses with Any Alterations	N(%)	11(7.5)	8(7.0)	10(6.4)	12(9.3)
Litters with Fetuses with Any Alterations	N(%)	8(44.4)	4(26.7)	8(44.4)	8(53.3)
Percent Fetuses per Litter with Alterations	MEAN	9.0	7.7	7.2	10.1
	SD	13.9	15.2	9.9	12.4
VARIATIONS[b]					
Fetuses with Any Variations	N(%)	9(6.1)	6(5.2)	10(6.4)	10(7.8)
Litters with Fetuses with Any Variations	N(%)	7(38.9)	2(13.3)	8(44.4)	7(46.7)
Percent Fetuses per Litter with Variations	MEAN	7.8	4.6	7.2	8.9
	SD	13.7	13.4	9.9	12.3
MALFORMATIONS[c]					
Fetuses with Any Malformations	N(%)	2(1.4)	2(1.7)	0(0.0)	3(2.3)
Litters with Fetuses with Any Malformations	N(%)	2(11.1)	2(13.3)	0(0.0)	3(20.0)
Percent Fetuses per Litter with Malformations	MEAN	1.1	3.2	0.0	2.3
	SD	3.3	9.1	0.0	5.1

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data. Fisher's Exact Test was used for proportion data.

a - All percentages were calculated on the basis of the number of live fetuses in each group.
b - Common observations in this species and strain and reversible delays or accelerations in development.
c - Irreversible changes occurring at low incidences in this species and strain.

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TABLE 9

BMS-214778: ORAL STUDY OF EMBRYO-FETAL DEVELOPMENT IN RABBITS

SUMMARY OF FETAL GROSS EXTERNAL AND PLACENTAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-214778 5	3 BMS-214778 30	4 BMS-214778 200
FETUSES EVALUATED	N	147	115	157	129
Live Fetuses	N	147	115	157	129
Dead Fetuses	N	0	0	0	0
LITTERS EVALUATED	N	18	15	18	15
PALATE: Arched (V)					
Fetal Incidence	N(%)	0	0	0	1(0.8)
Litter Incidence	N(%)	0	0	0	1(6.7)
BACK: Spina bifida (M)					
Fetal Incidence	N(%)	0	0	0	1(0.8)
Litter Incidence	N(%)	0	0	0	1(6.7)
FOREPAW(S): Malrotated (M)					
Fetal Incidence	N(%)	0	0	0	1(0.8)
Litter Incidence	N(%)	0	0	0	1(6.7)
FORE- AND HINDPAW(S): Digits, fused (M)					
Fetal Incidence	N(%)	0	1(0.9)	0	0
Litter Incidence	N(%)	0	1(6.7)	0	0
HINDLIMB(S) AND HINDPAW(S): Discolored and misshapen (V)					
Fetal Incidence	N(%)	0	0	0	1(0.8)
Litter Incidence	N(%)	0	0	0	1(6.7)

(M) = Malformation (V) = Variation

a - All percentaged were calculated on the basis of live fetuses in each group. There were no remarkable placental observations.

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STUDY NO. 99001

TABLE 11

BMS-214778: ORAL STUDY OF EMBRYO-FETAL DEVELOPMENT IN RABBITS

SUMMARY OF FETAL SKELETAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-214778 5	3 BMS-214778 30	4 BMS-214778 200
FETUSES EVALUATED	N	147	115	157	129
Live Fetuses	N	147	115	157	129
Dead Fetuses	N	0	0	0	0
LITTERS EVALUATED	N	18	15	18	15
PUBES: Incomplete ossification (V)					
Fetal Incidence	N(%)	0	0	0	4(3.1)*
Litter Incidence	N(%)	0	0	0	3(20.0)
HYOID: Alae, angulated (M)					
Fetal Incidence	N(%)	2(1.4)	0	2(1.3)	1(0.8)
Litter Incidence	N(%)	2(11.1)	0	2(11.1)	1(6.7)
HYOID: Body, incomplete ossification (V)					
Fetal Incidence	N(%)	4(2.7)	6(5.2)	3(1.9)	1(0.8)
Litter Incidence	N(%)	2(11.1)	2(13.3)	3(16.7)	1(6.7)
STERNEBRAE: Hyperplastic, cleaved, or asymmetric (V)					
Fetal Incidence	N(%)	2(1.4)	0	4(2.5)	3(2.3)
Litter Incidence	N(%)	2(11.1)	0	4(22.2)	3(20.0)
RIBS: Fused (M)					
Fetal Incidence	N(%)	1(0.7)	0	0	0
Litter Incidence	N(%)	1(5.6)	0	0	0
RIBS: 7th cervical, present (M)					
Fetal Incidence	N(%)	0	1(0.9)	0	0
Litter Incidence	N(%)	0	1(6.7)	0	0
VERTEBRAE: Centra, incompletely or not ossified (V)					
Fetal Incidence	N(%)	0	0	1(0.6)	1(0.8)[b]
Litter Incidence	N(%)	0	0	1(5.6)	1(6.7)
VERTEBRAE: Arches, bifid (V)					
Fetal Incidence	N(%)	0	0	0	1(0.8)[b]
Litter Incidence	N(%)	0	0	0	1(6.7)
VERTEBRAE: Arches, fused (M)					
Fetal Incidence	N(%)	0	0	0	1(0.8)[b]
Litter Incidence	N(%)	0	0	0	1(6.7)

(M) = Malformation (V) = Variation

Statistical Analysis: Fisher=s Exact test.
* Significantly different from control at P≤0.05.

a - All percentages were calculated on the basis of the number of live fetuses in each group.
b - Observed in fetus 4F0071-L3.

9.3 Prenatal and Postnatal Development

TAJ0015: Tasimelteon: PRELIMINARY STUDY FOR EFFECTS ON PRE- AND POST-NATAL DEVELOPMENT STUDY IN THE CD RAT BY ORAL GAVAGE ADMINISTRATION
Conducted by [redacted] (b) (4) non-GLP, initiated 6/20/11
Final report issued 7/12/12, Amendment dated 10/19/12

Species: Female Crl:CD(SD) rats
Drug: Tasimelteon (VEC-162), [redacted] (b) (4), batch 800167140, 99.8% pure
Dosing/Formulation: in 100% PEG-400, PO (gavage), QD

Table 2: Groups and dose levels and identification of the F0 generation

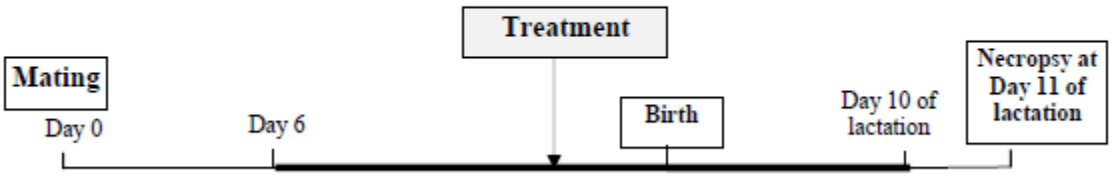
Group	Treatment	Dose# (mg/kg/day)	Number of females	Animal numbers
1	Control	0	6	1-6
2	Tasimelteon	50	6	7-12
3	Tasimelteon	150	6	13-18
4	Tasimelteon	450	6	19-24

Test material was used as supplied

Table 3: Formulation concentrations and dose volumes

Group	Treatment	Dose (mg/kg/day)	Concentration (mg/mL)	Volume dose (mL/kg)
1	Control	0	0	2.5
2	Tasimelteon	50	20	2.5
3	Tasimelteon	150	60	2.5
4	Tasimelteon	450	180	2.5

Study Design



In [redacted] (b) (4) Study TAJ0001, tasimelteon (VEC-162) in PEG-400 formulations (7.5 and 100 mg/mL) were found to be homogeneous and stable for 24 hours at ambient temperature, and for up to 15 days when refrigerated. The homogeneity and stability of tasimelteon in PEG 400 formulations at 200 mg/mL was performed as part of the ongoing pivotal pre- and post-natal study ([redacted] (b) (4) Study TAJ0017).

One HD dam (#19) was killed on GD25 after failing to deliver a litter. This dam appeared to have aborted (blood in the cage on GD22; no pups apparent); one placenta was observed in the uterus at necropsy. Drug-related clinical signs after dosing the dams included: chin rubbing, salivation, excessive chewing, underactivity, and piloerection, as well as pica (eating feces; MD and HD), “muscle reaction- unsteady” (HD), partially closed eyelids (HD), and flat posture (HD). One HD dam (#23) was observed to show pale feces on GD12. Body weight gains were reduced 36% at HD, compared to controls, from GD6-14; afterward, gains were similar or increased throughout gestation and lactation (e.g. tasimelteon-treated dams gains increased approximately 20% compared to controls LD1-11). Food consumption was reduced up to 12 and 35% in MD and HD dams, respectively, at the beginning of dosing (GD6 to GD10 or GD14, respectively); afterward, consumption was similar throughout gestation and lactation. All dams delivered after a normal gestation period (22-23 days), but HD dams showed a very slightly increased gestation length. The sponsor reported no drug-related macroscopic changes.

The sponsor reported no tasimelteon-related adverse effect on group mean number of implantations, litter size, fetal survival, fetal clinical signs, or fetal necropsy; however, the calculated values exclude data from the HD dam that did not deliver (presumed abortion). For this reason, an effect on fetal survival cannot be excluded. Additionally, a few pups from treated dams did not survive. One male LD pup was missing on D4. One LDF pup, one MDM pup, and one HDF pup were found dead between birth and LD1. Both the LDF and HDF pups had no milk present in the stomach (the LDF pup also showed an unexpanded lung). One HDF pup was pale on LD1. According to the sponsor, sex ratios (i.e., % males) at birth were slightly high in the Con and LD groups and slightly reduced in the MD and HD groups. Mean fetal body weights were reduced (approximately 5 to10%) at MD and HD, both at LD1 and LD11 (difference greater at LD11).

The sponsor indicated that tasimelteon treatment was believed to have “delayed fetal development in utero and also reduced the rate of growth postpartum” at HD; a similar fetal effect was observed at MD. Based on the results of this study, doses of 0, 50, 150, and 400 mg/kg were selected for the pivotal pre-/post-natal development study.

Reference ID: 3399188

Study title: TASIMELTEON: STUDY FOR EFFECTS ON PRE- AND POST-NATAL DEVELOPMENT IN THE CD RAT BY ORAL GAVAGE ADMINISTRATION	
Study no.:	TAJ0017
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/24/11
GLP compliance:	Yes (b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	tasimelteon (VEC-162), (b) (4) batch 800167140, 100.4% pure (w/w)

Methods	
Frequency of dosing:	QD
Dose volume:	2.5 mL/kg
Route of administration:	PO (oral gavage)
Formulation/Vehicle:	100% polyethylene glycol-400 (PEG-400)
Species/Strain:	Crl:CD(SD) rats, females
Study design:	See sponsor's table, below
Deviation from study protocol:	None identified.

Table 2: Groups and dose levels and identification of the F0 generation

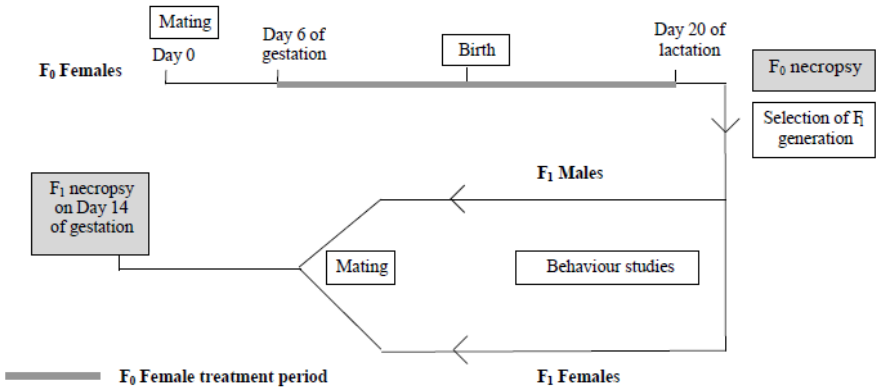
Group	Treatment	Dose# (mg/kg/day)	Number of females	Animal numbers
1	Control	0	22	1-22
2	Tasimelteon	50	22	23-44
3	Tasimelteon	150	22	45-66
4	Tasimelteon	450	22	67-88

Expressed in terms of test material as supplied.

Table 3: Groups and identification of the F1 generation

Group	Number of animals		Animal numbers	
	Male	Female	Male	Female
1	20	20	101-120	181-200
2	20	20	121-140	201-220
3	20	20	141-160	221-240
4	20	20	161-180	241-260

7.1.3. Study Design



Observations and Results

F_0 Dams

Survival: There were no F0 mortalities.

Clinical signs: Few dose-related clinical signs were observed. After dosing, chin rubbing and/or salivation was observed in most VEC-162-treated dams. Convulsion (1 HD; see description below), partially closed eyelids (6 HD), unresponsive (3 HD), flat posture (2 HD), breathing fast (1 HD), limited use of limbs (1 HD), and underactive (5 MD and 22 HD, LD5 to end), and/or unsteady gait (2 MD and 20 HD, for 1-3 days on average; see sponsor's definition below) were observed after dosing. During the detailed physical examinations, hair loss, piloerection, pale feces, irritable behavior, and /or vocalization were observed with increased incidence during the gestation or lactation periods in a few VEC-treated animals.

One HD dam was observed to have a 30 second convulsion on LD15; this dam was previously observed to be underactive, unresponsive, and have flat posture shortly after dosing on LD8, underactive on LD12 and LD19, and underactive with unsteady muscle reaction ("lack of muscular coordination giving rise to staggering or otherwise abnormal gait") on LD15.

Body weight: During gestation, average body weights were very slightly reduced in MD and HD dams (see sponsor's Figure 1, below; ~3-5%). Overall average body weight was reduced (3 to 5%) in HD dams throughout gestation. Average body weight gain at HD was decreased 83% from GD6 to GD10 and was reduced 15% overall (GD6-GD20). At MD, average body weight gain was reduced approximately 30% from GD6 to GD10, but was similar to controls (-5%) during the remainder of the gestation period. There were no clear differences in LD dams.

During lactation, average body weights were generally slightly lower than those of controls (<5%); HD dams did not show the loss exhibited by the other groups at the end of the lactation period (LD18-LD21), which generated a net increase in body weight gain over the lactation period at HD (~30%).

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Figure 1: Bodyweight during gestation

Bodyweight - group mean values (g) for females during gestation (F0)

Group	1	2	3	4
Compound	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	0	50	150	450

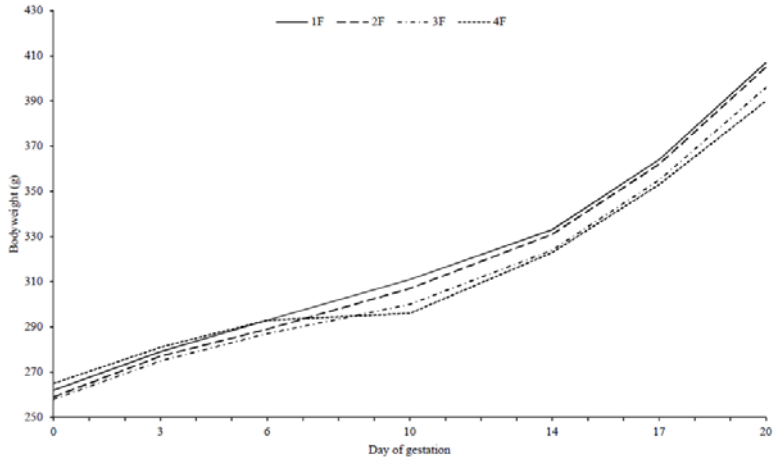
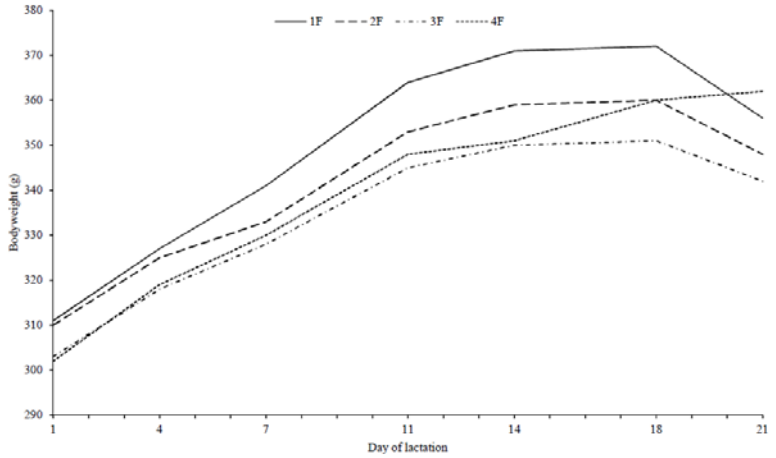


Figure 2: Bodyweight during lactation

Bodyweight - group mean values (g) for females during lactation (F0)

Group	1	2	3	4
Compound	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	0	50	150	450



Food consumption: Food consumption was reduced 15% on GD6 to GD9 in MD dams and throughout the gestation period in HD dams (35% GD6-GD9, average reduction 16% GD6-GD19). Food consumption was also reduced (7-13%) during LD7 to LD17 at MD and HD.

Uterine content: Gestation length was within the expected length (22-33.5 days) for all groups, but the mean gestation length was slightly increased in tasimelteon-treated groups (dose-related, [ss]). Two LD dams were found not pregnant, and one LD dam was sacrificed on GD23 after showing total litter loss "shortly after the commencement of parturition." However, a relationship to drug cannot be established since similar events did not occur at MD or HD.

There were 22, 19, 22, and 22 litters for examination in Control, LD, MD, and HD groups. The sponsor reported no drug-related effects on the number of implantations or litter size (but note that data from the LD#32 with total litter loss were removed). LD#32 had 14 implantation sites; this dam was coded as "no live litter" and the sponsor stated that "most offspring were dead or presumed eaten at parturition."

Necropsy observation: The sponsor reported no drug-related macroscopic changes. Increased hair loss was reported in tasimelteon-treated dams. Two HD dams showed kidney changes (punctate depressions, pelvic dilatation). One HD dam showed multiple punctate dark areas on the thymus. A few changes of unclear toxicological significance observed in ovaries (cysts), mammary gland (pale, inactive), and spleen (enlarged) were observed only at LD.

Toxicokinetics: Plasma exposures to tasimelteon (VEC-162), and metabolites M9, M12, M13, and M14 were evaluated at GD6, GD17 and LD4. Repeat analyses were necessary for a number of samples for M12 and M13; one sample for M14 was repeated.

Control animals showed some exposure to tasimelteon and/or its metabolites, but were "generally close to or below the limit of quantitation" (i.e., < 5.438 ng/mL for M14 and < 5 ng/mL for all others). Low level exposures were also observed in samples from offspring animals (5.07 to 15.5 ng/mL). After an investigation, the sponsor attributed these observations to contamination during sample handling.

See sponsor's Tables 68-72 for summary plasma exposure data in dams and Tables 73-76 for summary plasma exposure data in the offspring.

Reference ID: 3399188

Plasma exposures to tasimewlteon were generally less than dose-proportional (see sponsor's Tables 68 and 11), and little to no accumulation was observed in the dams (see sponsor's Table 12). There was a >100-fold margin from the plasma tasimelteon AUC at the LD to the AUC following a 20 mg dose in humans (~411 ng·hr/mL).

Table 68 Pharmacokinetic parameters of tasimelteon on Day 6 and Day 17 of gestation (Days 1 and 12 of treatment) following daily oral gavage administration of tasimelteon to pregnant female rats

Day 6

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k (hours^{-1})	t½ (hours)
50	7240	8	-	65800	a	-
150	10400	2	1980	137000	0.0962	7.2
450	16300	2	7750	228000	a	-

Day 17

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k (hours^{-1})	t½ (hours)
50	4720	2	-	44000	a	-
150	12600	2	-	123000	a	-
450	18200	8	6450	256000	a	-

a Terminal rate constant could not be adequately estimated

Table 11 C_{max} and AUC_{0-24h} ratios of tasimelteon following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Dose level ratio	C_{max} ratio		AUC_{0-24h} ratio	
		Day 6	Day 17	Day 6	Day 17
50	1	1	1	1	1
150	3.0	1.4	2.7	2.1	2.8
450	9.0	2.3	3.9	3.5	5.8

Table 12 Accumulation ratios of tasimelteon following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Accumulation ratio
50	0.67
150	0.90
450	1.1

Plasma exposures to major metabolite M9 were generally less than dose-proportional (see sponsor's Tables 69 and 14); however accumulation was observed at HD (see sponsor's Table 15).

Table 69 Pharmacokinetic parameters of M9 on Day 6 and Day 17 of gestation (Days 1 and 12 of treatment) following daily oral gavage administration of tasimelteon to pregnant female rats

Day 6

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	573	2	-	5360	a	-
150	948	2	380	10800	a	-
450	1120	2	645	14700	a	-

Day 17

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	503	2	11.8	4430	0.1889	3.7
150	1350	2	15.9	11100	0.2362	2.9
450	2160	8	777	29800	a	-

a Terminal rate constant could not be adequately estimated

Table 14 C_{max} and AUC_{0-24h} ratios of M9 following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Dose level ratio	C_{max} ratio		AUC_{0-24h} ratio	
		Day 6	Day 17	Day 6	Day 17
50	1	1	1	1	1
150	3.0	1.7	2.7	2	2.5
450	9.0	2.0	4.3	2.7	6.7

Table 15 Accumulation ratios of M9 following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Accumulation ratio
50	0.83
150	1.0
450	2.0

Plasma exposures of major metabolite M12 were generally less than dose-proportional (see sponsor's Tables 70 and 18), and accumulation was not observed (see sponsor's Table 19). Metabolite M12 plasma exposures were up to 3 times that of parent at LD, 2 times that of parent at MD and 1-2 times that of parent at HD.

Table 70 Pharmacokinetic parameters of M12 on Day 6 and Day 17 of gestation (Days 1 and 12 of treatment) following daily oral gavage administration of tasimelteon to pregnant female rats

Day 6

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	13700	8	-	184000	a	-
150	21200	12	10200	317000	a	-
450	20400	24	20400	414000	a	-

Day 17

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	12400	8	-	146000	a	-
150	19100	4	-	286000	a	-
450	25000	8	10500	397000	a	-

a Terminal rate constant could not be adequately estimated

Table 18 C_{max} and AUC_{0-24h} ratios of M12 following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Dose level ratio	C_{max} ratio		AUC_{0-24h} ratio	
		Day 6	Day 17	Day 6	Day 17
50	1	1	1	1	1
150	3.0	1.5	1.5	1.7	2
450	9.0	1.5	2.0	2.3	2.7

Table 19 Accumulation ratios of M12 following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Accumulation ratio
50	0.79
150	0.90
450	0.96

Plasma exposures of major metabolite M13 were generally less than dose-proportional (see sponsor's Tables 71 and 22), and some accumulation was observed only at HD (see sponsor's Table 23).

Table 71 Pharmacokinetic parameters of M13 on Day 6 and Day 17 of gestation (Days 1 and 12 of treatment) following daily oral gavage administration of tasimelteon to pregnant female rats

Day 6

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	89.4	8	-	883	a	-
150	67.1	12	56.8	1340	a	-
450	202	24	202	3200	a	-

Day 17

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	70.9	2	-	572	a	-
150	136	4	-	1400	a	-
450	279	8	199	5120	a	-

a Terminal rate constant could not be adequately estimated

Table 22 C_{max} and AUC_{0-24h} ratios of M13 following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Dose level ratio	C_{max} ratio		AUC_{0-24h} ratio	
		Day 6	Day 17	Day 6	Day 17
50	1	1	1	1	1
150	3.0	0.8	1.9	1.5	2.4
450	9.0	2.3	3.9	3.6	9.0

Table 23 Accumulation ratios of M13 following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Accumulation ratio
50	0.65
150	1.0
450	1.6

Plasma exposures of major metabolite M14 were generally less than dose-proportional (see sponsor's Tables 71 and 26), and some accumulation was observed only at HD (see sponsor's Table 27).

Table 72 Pharmacokinetic parameters of M14 on Day 6 and Day 17 of gestation (Days 1 and 12 of treatment) following daily oral gavage administration of tasimelteon to pregnant female rats

Day 6

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	551	8	-	6380	a	-
150	911	8	183	12700	0.1045	6.6
450	891	8	793	17900	a	-

Day 17

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	512	4	-	5090	a	-
150	976	8	-	14000	a	-
450	1890	8	816	29300	a	-

a Terminal rate constant could not be adequately estimated

Table 26 C_{max} and AUC_{0-24h} ratios of M14 following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Dose level ratio	C_{max} ratio		AUC_{0-24h} ratio	
		Day 6	Day 17	Day 6	Day 17
50	1	1	1	1	1
150	3.0	1.7	1.9	2.0	2.8
450	9.0	1.6	3.7	2.8	5.8

Table 27 Accumulation ratios of M14 following oral gavage administration of tasimelteon to pregnant female rats

Dose level (mg/kg/day)	Accumulation ratio
50	0.80
150	1.1
450	1.6

Dosing Solution Analysis: Formulations were prepared weekly and stored refrigerated (2-8°C) before use. Homogeneity and stability at 7.5 and 100 mg/mL were established in Study TAJ0001; homogeneity and stability at 200 mg/mL was confirmed in this study. Suspensions were homogenous and stable for 8 days at ambient temperature and for up to 15 days refrigerated. The mean concentrations were within 3% of nominal.

F_1 Generation

Survival: The sponsor reported no drug-related effects on litter size, survival indices, or sex ratios; however, there was a slight reduction in the live birth index at HD (see sponsor's Table 49, below). No milk was present in the stomach of 4, 1, 2, and 11 fetuses in the control, LD, MD, and HD groups (presumed stillborn; from 4, 1, 2, and 5 litters, respectively). One deceased HD fetus was noted to be of "small build" with bruising to the nares. Also, the live birth index should be adjusted for the sponsor's removal of data from LD#32 (14 implantations, "no live litter" although the individual line data show 1 male and 3 female pups at delivery; est. adjusted live birth index ~94.6%).

Table 49: Offspring survival indices

Offspring survival indices - group mean values (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

Group		Post implantation survival index (%)	Live birth index (%)	Viability index (%)	Lactation index (%) on Day 21
Statistical test:		Fe	Wi	Fe	
1	Mean	92.7	99.0	98.8	98.6
	N	22	22	22	22
2	Mean	91.0	99.6	98.9	99.5
	N	19	19	19	19
3	Mean	89.5	98.7	99.3	100.0
	N	22	22	22	22
4	Mean	91.4	96.5	99.4	100.0
	N	22	22	22	22

Fe Treated groups compared to Control using Fisher's Exact test.
Wi Treated groups compared to Control using Williams' test.

Clinical signs: For early observations of the F1 pups, the sponsor reported a low incidence of clinical signs, including: cold to touch, little milk in stomach, minor cuts, and bruising. In tasimelteon-treated groups, underactive, swollen area on the abdomen, small build were also observed in a few offspring.

Although the protocol defined how selection of the F1 mature animals would be performed (i.e., one/sex/litter), it is unclear how this selection was actually done. For example, there were 22 litters for the control and MD groups (i.e., 2 litters in each group were not represented) and, although there were 19 LD

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and 22 HD litters, the sponsor appears to have selected the representative F1 generation from 18 and 19 litters, respectively. The F1 mature animals were then numbered in such a way that one cannot determine from which litter each animal was born.

At maturation, few clinical signs were reported, including: broken teeth (2 LDM), vocalization (2 MDF), abnormal staining on head (1 MDF), swollen area in the ventral abdomen (1 MDM, 1 HDM), and kinked tail (1 HDM). During early gestation for the F1 dams, few clinical signs were reported, including: vocalization (2 LDF), irritable (1 LDF, 1 HDF), and abnormal staining on head (1 MDF).

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Body weight: Generally, average body weights of the F1 pups were reduced during the entire lactation period. However, LD and MD pups were slightly heavier than control pups at LD1, which the sponsor attributed to the slightly longer gestation lengths; although gestation length was increased 0.7 days for the HD pups, their average body weight on LD1 was 0.3 g less than that of control pups. The sponsor noted that the birth weight of the HD group was 0.8 g less than that of controls of similar gestation length, and stated that "there may have been a delay in pup growth in utero contributing to the extended gestation length." There was an incremental body weight gain reduction in the HD pups between LD4 and LD18 (-12% at weaning, compared to controls). Body weight gains over LD1 to LD21 were reduced 15% in the HD pups, and they weighed 14% less than controls at 25 days of age.

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Figure 3: Bodyweight for male offspring

Bodyweight - group mean values (g) for male offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

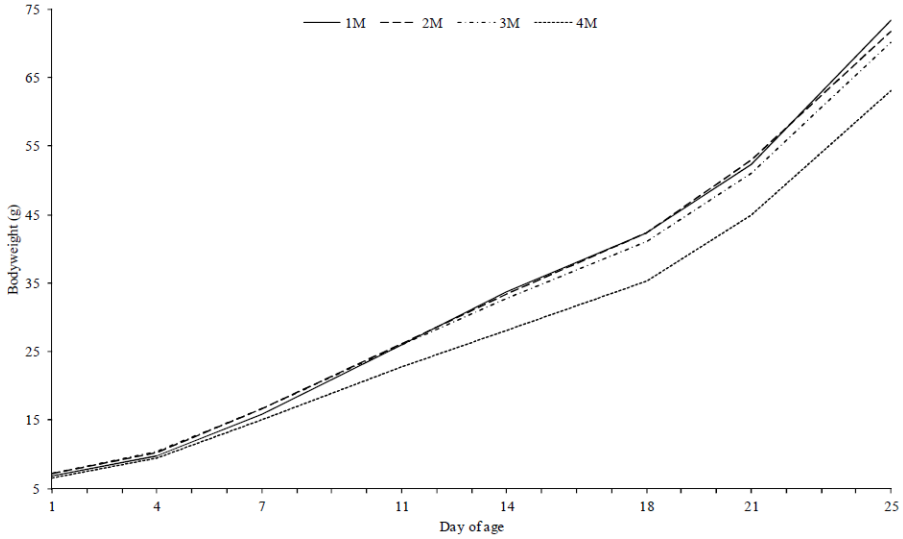
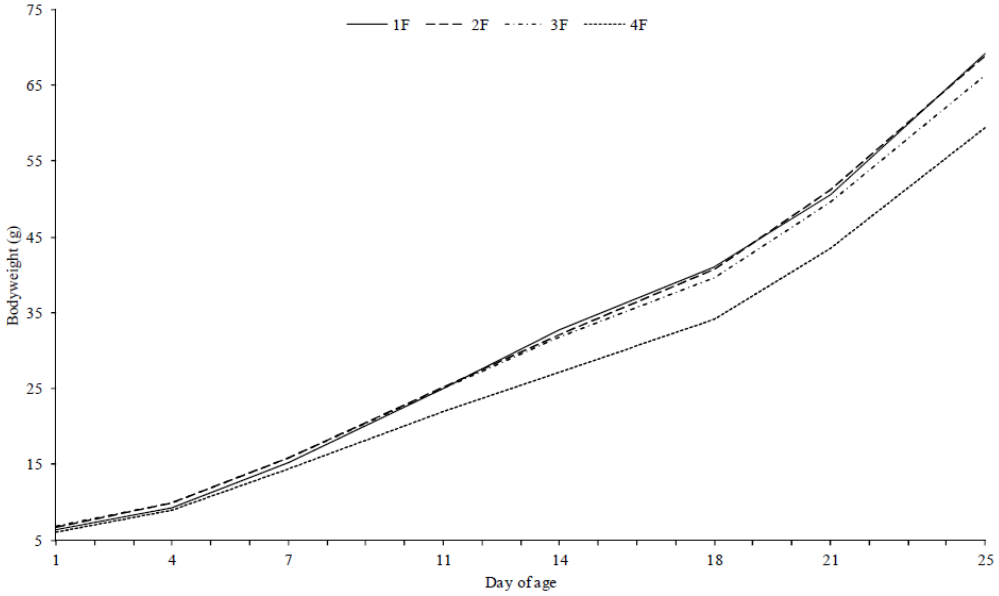


Figure 4: Bodyweight for female offspring

Bodyweight - group mean values (g) for female offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450



After weaning on LD21 (although the sponsor nominally defined the "start" of the F1 generation as 28 days of age), average body weights in the F1 animals continued to be reduced in the tasimelteon-treated groups, especially the HD group (see sponsor's Table 39, and Figures 8 and 9). Generally, average body weights in the LDM and MD groups were reduced 5 to 10 percent, and average body weights in the HD groups were reduced 10 to 20 percent. In the F1 females, this average body weight reduction continued during gestation (~5% at MD and ~10% at HD; see sponsor's figure 9).

Table 39: Weight gain of F1 generation

		Group 1	Group 2	Group 3	Group 4
		Control gain	% Control	% Control	% Control
Males	D1-60	413	94	95	90**
Females	D1-39	172	100	93*	92*
Females	Gestation D0-14	75	104	96	95

* $p<0.05$; ** $p<0.01$

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Figure 7: Bodyweight for males

Bodyweight - group mean values (g) for males (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

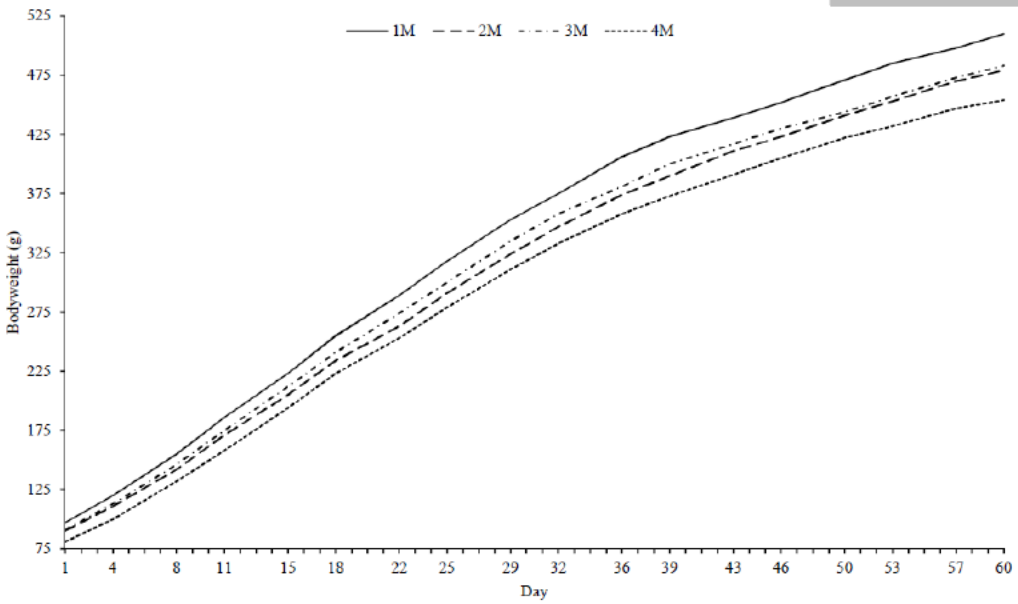

Figure 8: Bodyweight for females before pairing

Bodyweight - group mean values (g) for females before pairing (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

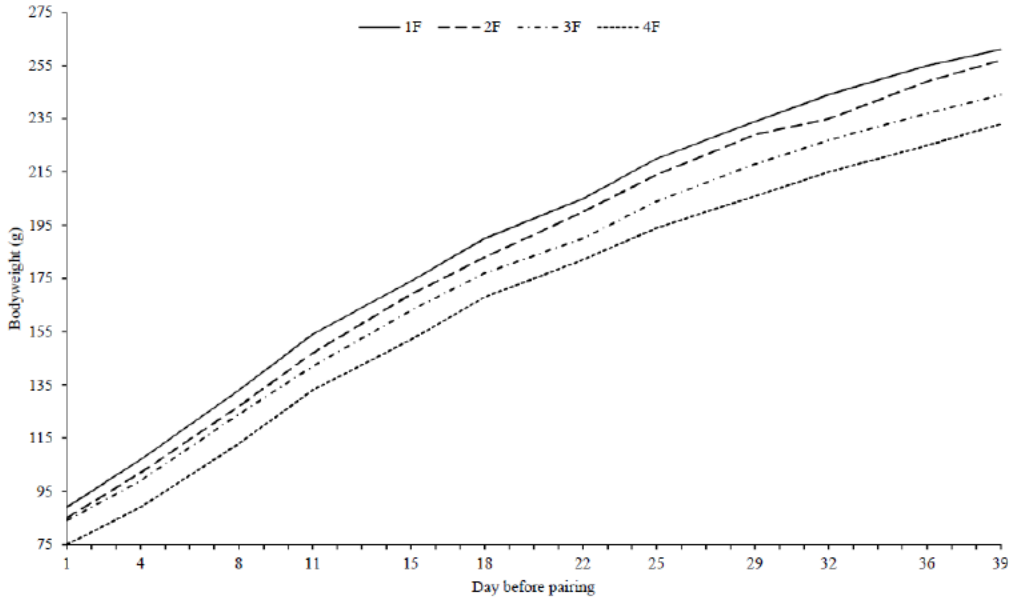

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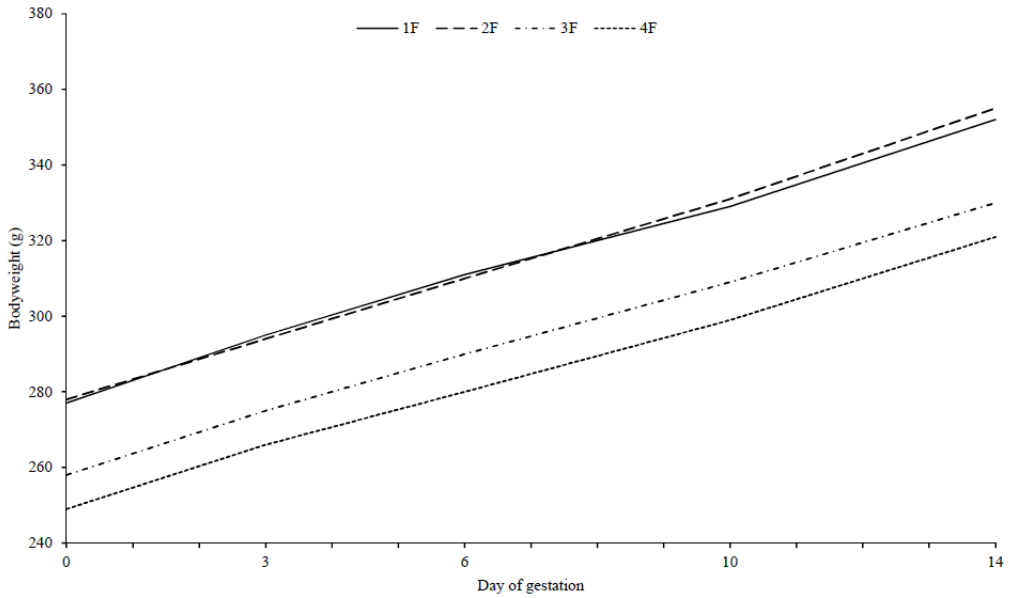
Figure 9: Bodyweight for females during gestation

Bodyweight - group mean values (g) for females during gestation (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450



Food consumption: Data not provided

Necropsy Observations: Of the pups dying prior to scheduled termination, there was an increase in no milk present in the stomach at HD (4/4, 1/1, 2/2, and 11/5 [pups/litter] in the control, LD, MD, and HD groups).

A few observations were noted in the F1 pups at scheduled termination. Small build was reported for 1/1 and 45/6 (pup/litter) at MD and HD, respectively. Dilated renal pelvis was observed in 4/4, 4/4, and 1/1 (pup/litter) in the LD, MD and HD groups, respectively. (Of unclear importance were 3 LD pups [1 litter] observed to show fluid-filled dilated ventricles in the brain.)

At maturation, few alterations were observed. In tasimelteon-treated males, alterations in testes (flaccid, blue, and/or small; 1 LDM, 2 HDM), epididymides (small; 1 LDM, 2 HDM), seminal vesicles (abnormal shape; 1 HDM), and tail (kinked; 1 HDM) were observed. Of unclear toxicological importance is 1 LDM that showed dilated ventricles in the brain. In tasimelteon-treated females, alterations in kidney (pelvic dilatation, enlarged, and/or dark; 1 LDF, 1 MDF), ureters (distended, thickened; 1 MDF), and urinary bladder (thickened, distended, dark, and calculus present; 1 MDF) were observed.

Physical development: Sexual maturation was assessed beginning at 28 days of age for females and 38 days of age for males. Mean age at balano-preputial separation for males was not clearly affected, but it was achieved at a lower average body weight at HD. Mean age at vaginal opening for females was increased at HD (although body weight was similar to that of controls); the sponsor stated that "sexual maturation was occurring when the females reached a critical body weight." See sponsor's summary Table 56 for details.

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Table 56: Balano-preputial separation and vaginal opening

Balano-preputial separation and vaginal opening - group mean age and bodyweight at attainment for selected offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

Group		Time of completion for balano preputial separation Day of age	Bodyweight (g) at balano preputial separation	Time of completion for vaginal opening Day of age	Bodyweight (g) at vaginal opening
Statistical test:		Wi	Wi	Wi	Wi
1	Mean	45	249	34	121
	SD	2.7	27.0	2.2	14.8
	N	20	20	20	20
2	Mean	47	243	34	121
	SD	2.8	28.5	2.2	13.5
	N	20	20	20	20
3	Mean	46	238	35	117
	SD	2.5	24.3	1.3	9.9
	N	20	20	20	20
4	Mean	46	227*	36**	120
	SD	2.4	26.6	1.6	14.0
	N	19	19	20	20

Wi Treated groups compared to Control using Williams' test.
* $p<0.05$
** $p<0.01$

Neurological assessment: The HD pups demonstrated an increased age to achieve surface righting (assessed from LD2 until achieved) and air righting (assessed from LD14 until achieved), compared to controls; see the sponsor's summary Table 55, below. Additionally, an increased number of HD pups failed to achieve surface and/or air righting within the allotted time period (4, 1, 2, and 11 in the control, LD, MD and HD groups, respectively); data for these pups were not accounted for by the sponsor's method of reporting the summary data. The sponsor reported no drug-related effect on pupil reflex and startle response on LD20 (as assessed by % of animals passed). Two LD pups showed no or partial pupil dilation; this was not reported in other groups.

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Table 55: Pre-weaning examinations

Pre-weaning examinations - group mean values for offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

Group		Surface righting Day of age	Air righting Day of age		Pupil reflex (% pass)	Startle response (% pass)
Statistical test:		Wi	Sh			
1	Mean	3.4	15.9	Number of animals tested	216	216
	SD	0.73	0.37	Number of animals failed	0	0
	N	22	22	% of animals passed	100.0	100.0
2	Mean	3.2	15.9	Number of animals tested	187	187
	SD	0.71	0.56	Number of animals failed	1	0
	N	19	19	% of animals passed	99.5	100.0
3	Mean	3.3	15.9	Number of animals tested	218	218
	SD	0.71	0.79	Number of animals failed	0	0
	N	22	22	% of animals passed	100.0	100.0
4	Mean	3.9*	16.5*	Number of animals tested	220	220
	SD	0.81	0.73	Number of animals failed	0	0
	N	22	22	% of animals passed	100.0	100.0

Sh Treated groups compared to Control using Shirley's test.
Wi Treated groups compared to Control using Williams' test.
* $p<0.05$

Motor activity testing was assessed at LD22, and showed no consistent drug-related effects (see sponsor's Figure 5, below). While an apparent slight reduction in beam breaks in the MD and HD males started at 42-48 minutes, compared to controls, it is of questionable toxicological importance given that the SDs at these times were approximately equal to the averages. Likewise, tasimelteon-treated females were slightly more active during the first 6-12 minutes, but averages were within 1 SD.

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Figure 5: Motor activity

Motor activity - group mean scores (beam breaks) for male offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

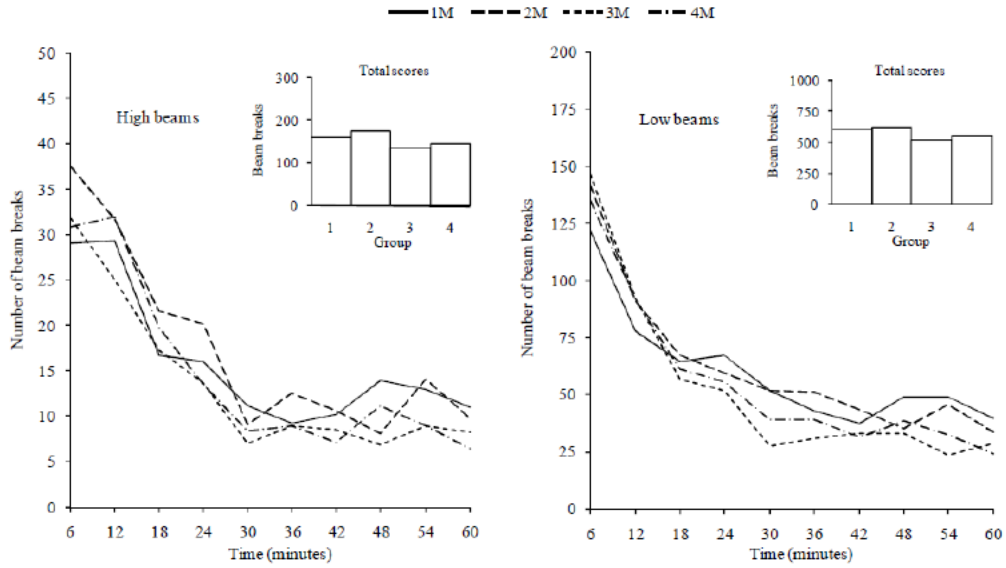
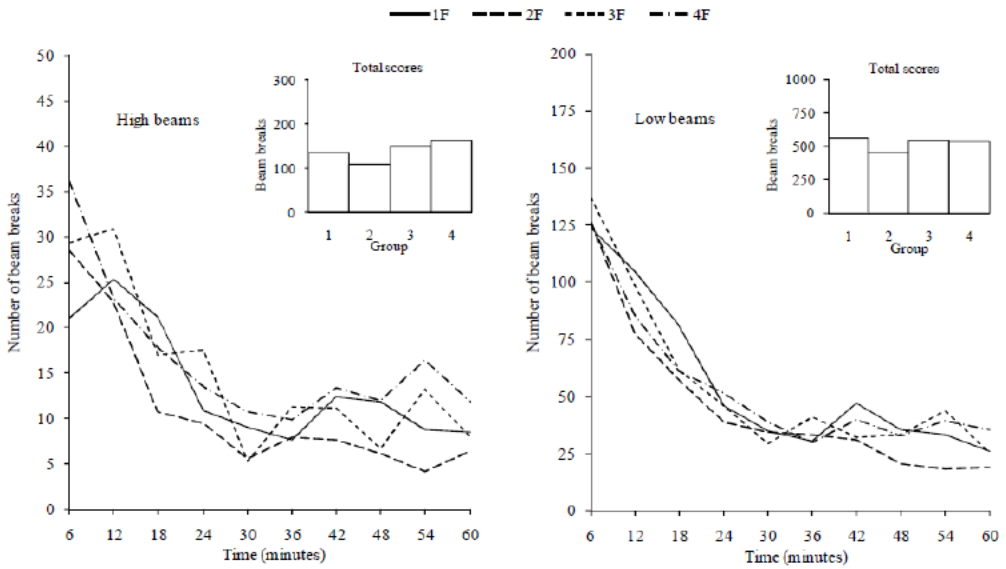


FIGURE 5 - continued

Motor activity - group mean scores (beam breaks) for female offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450



At 31±1days of age (and again at 48± 1 days of age for HDM), Morris Water maze performance was assessed. F1 HDM demonstrated clear deficits (see sponsor's summary Table 58 and Figure 6). Trial times, sector entries, and failed trials were increased for HDM on all four testing days. While the day-to-day testing showed improvements similar to

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those of controls, the absolute values remained consistently higher than those of controls and many of the absolute values were outside of the historical control range. The MDM showed deficits in performance on the first day of testing, but subsequent performance was similar to that of controls. The performance of F1 females was not affected.

Table 58: Morris maze performance

Morris maze performance - group mean values for offspring at Day 30-32 of age (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

Group /Sex	Number of animals		Day 1 T	Day 1 F	Day 1 S	Day 2 T	Day 2 F	Day 2 S	Day 3 T	Day 3 F	Day 3 S	Day 4 T	Day 4 F	Day 4 S
Statistical test:			Wi	Sh	Wi	Wi	Fe	Wi	Wi	Fe	lWi	lWi	Fe	lWi
1M	20	Mean	50.7	0.9	17.3	25.3	0.3	10.1	15.5	0.0	6.6	8.4	0.0	4.1
		SD	17.1	0.7	5.7	19.9	0.6	6.3	9.7	0.0	3.5	4.2	0.0	1.9
		%		75.0			20.0			0.0			0.0	
2M	20	Mean	47.9	0.8	16.2	30.4	0.5	12.2	15.2	0.1	6.1	11.6	0.0	5.6
		SD	23.6	1.1	6.5	24.4	1.0	7.9	7.9	0.2	2.2	7.0	0.0	3.7
		%		45.0			20.0			5.0			0.0	
3M	20	Mean	61.9	1.4	21.3	27.3	0.2	10.8	15.0	0.1	6.5	12.6	0.0	5.9
		SD	23.1	1.1	7.9	17.9	0.4	6.0	8.4	0.2	3.2	7.3	0.0	3.9
		%		75.0			15.0			5.0			0.0	
4M	20	Mean	62.9	1.6*	21.5	34.2	0.6	13.8	21.9	0.1	10.2*	18.7**	0.2	8.8**
		SD	18.3	0.9	4.9	26.8	1.0	9.2	14.0	0.2	6.0	15.2	0.5	6.4
		%		95.0			30.0			5.0			10.0	

T Trial time (seconds - mean of 3 trials)
F Number of failed trials (90 seconds)
S Sector entries (mean of 3 trials)
% Percentage of animals with at least 1 failed trial (90 seconds)
l Data were log transformed for the statistical analysis.
Fe Treated groups compared to Control using Fisher's Exact test.
Sh Treated groups compared to Control using Shirley's test.
Wi Treated groups compared to Control using Williams' test.
* $p<0.05$
** $p<0.01$

Morris maze performance - group mean values for offspring at Day 30-32 of age (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

Group /Sex	Number of animals		Day 1 T	Day 1 F	Day 1 S	Day 2 T	Day 2 F	Day 2 S	Day 3 T	Day 3 F	Day 3 S	Day 4 T	Day 4 F	Day 4 S
Statistical test:			Wi	Sh	Wi	Wi	Fe	Du	Wi	Fe	Wi	lWi	Fe	Wi
1F	20	Mean	57.8	1.2	19.2	35.5	0.4	14.0	14.7	0.0	6.1	11.2	0.0	5.3
		SD	23.4	1.2	6.3	19.8	0.6	7.0	8.0	0.0	2.7	5.5	0.0	2.1
		%		60.0			35.0			0.0			0.0	
2F	20	Mean	50.7	0.8	16.7	18.5	0.2	7.2**	17.7	0.1	7.2	12.3	0.0	5.0
		SD	16.0	0.8	5.0	14.9	0.5	4.0	10.0	0.3	3.3	8.0	0.0	2.7
		%		60.0			10.0			10.0			0.0	
3F	20	Mean	53.1	0.9	18.6	24.6	0.1*	10.3	12.5	0.0	5.6	14.5	0.1	6.6
		SD	21.5	1.0	6.2	16.8	0.4	4.5	7.5	0.0	3.5	11.0	0.2	4.3
		%		60.0			5.0			0.0			5.0	
4F	20	Mean	46.9	0.8	16.2	28.6	0.2	12.0	12.9	0.0	5.9	11.8	0.0	5.6
		SD	19.6	1.0	6.1	20.3	0.7	6.9	5.2	0.0	1.9	5.9	0.0	2.8
		%		55.0			10.0			0.0			0.0	

T Trial time (seconds - mean of 3 trials)
F Number of failed trials (90 seconds)
S Sector entries (mean of 3 trials)
% Percentage of animals with at least 1 failed trial (90 seconds)
l Data were log transformed for the statistical analysis.
Du Treated groups compared to Control using Dunnett's test.
Fe Treated groups compared to Control using Fisher's Exact test.
Sh Treated groups compared to Control using Shirley's test.
Wi Treated groups compared to Control using Williams' test.
* $p<0.05$
** $p<0.01$

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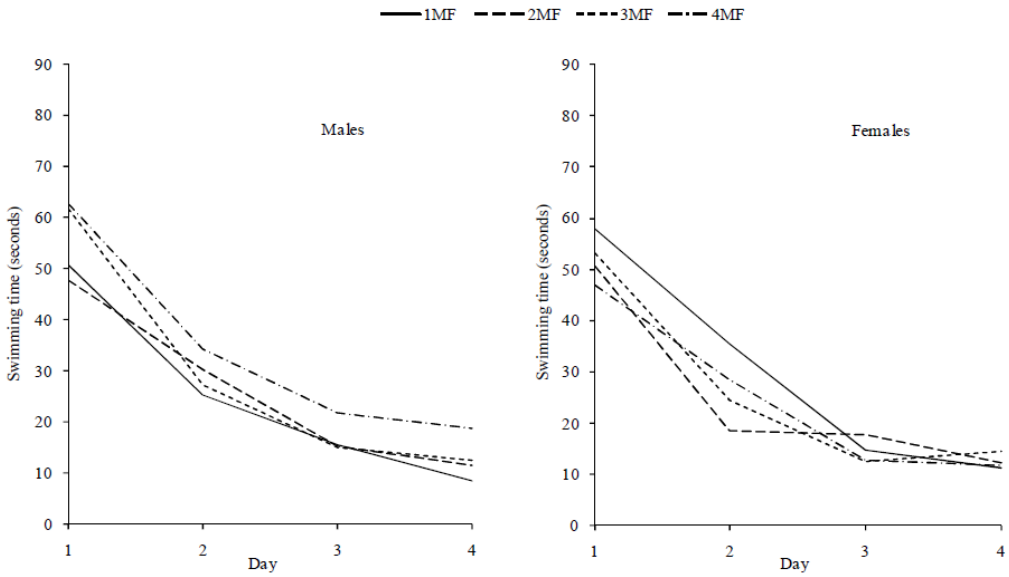
Figure 6: Morris maze performance

Morris maze performance - group mean times for offspring at Day 30-32 of age (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450



Although not standard practice (and not encouraged due to known effects of repeated testing), the sponsor re-tested the F1 males at 47 to 49 days of age to determine whether the observed deficits would resolve. While some inter-group variation remained in the performance of F1 males, the sponsor reported no drug-related consistent differences (see sponsor's continuation of Table 58, below).

Morris maze performance - group mean values for offspring at Day 47-49 of age (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

Group /Sex	Number of animals		Day 1 T	Day 1 F	Day 1 S	Day 2 T	Day 2 F	Day 2 S	Day 3 T	Day 3 F	Day 3 S	Day 4 T	Day 4 F	Day 4 S
Statistical test:			lWi	Fe	lWi	Wi		Wi	lWi		lWi	lWi		lWi
1M	20	Mean	13.8	0.0	5.4	11.4	0.0	4.8	9.4	0.0	3.9	6.2	0.0	2.8
		SD	6.7	0.0	2.6	5.7	0.0	2.3	6.1	0.0	2.0	2.2	0.0	0.8
		%		0.0			0.0			0.0			0.0	
2M	20	Mean	18.3	0.1	6.3	11.5	0.0	4.8	10.4	0.0	3.9	7.0	0.0	3.3
		SD	12.6	0.2	4.0	6.4	0.0	2.4	10.4	0.0	2.6	4.4	0.0	1.5
		%		5.0			0.0			0.0			0.0	
3M	20	Mean	15.8	0.0	5.9	10.0	0.0	4.3	6.5	0.0	2.9	8.9	0.0	3.7
		SD	7.0	0.0	2.0	7.5	0.0	2.7	2.3	0.0	0.9	6.4	0.0	2.0
		%		0.0			0.0			0.0			0.0	
4M	20	Mean	17.3	0.0	6.4	11.5	0.0	4.9	8.6	0.0	3.7	9.0	0.0	4.2**
		SD	6.7	0.0	2.1	5.3	0.0	1.9	3.8	0.0	1.4	5.0	0.0	2.2
		%		0.0			0.0			0.0			0.0	

T Trial time (seconds - mean of 3 trials)
F Number of failed trials (90 seconds)
S Sector entries (mean of 3 trials)
% Percentage of animals with at least 1 failed trial (90 seconds)
l Data were log transformed for the statistical analysis.
Fe Treated groups compared to Control using Fisher's Exact test.
Wi Treated groups compared to Control using Williams' test.
** $p<0.01$

Reproduction: After at least 9 weeks of age, mating pairs were assigned within the same treatment groups (with care taken to avoid sibling pairings). Mating pairs (one-to-one) were left together for up to 2 weeks. Pre-coital interval and fertility parameters were not clearly affected (see sponsor's Tables 65 and 66, below). Three LDF and one HDF were not pregnant; additionally, another HDF had been pregnant (#257; as indicated by a single resorption), but had no viable implants.

Table 65: Pre-coital interval

Pre-coital interval - group values (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

Group	Number of animals		Pre-coital interval (days) 1-4	5-8	9-12	13-14
1	20	N	20	0	0	0
		(%)	(100)			
2	20	N	19	0	0	1
		(%)	(95)			(5)
3	20	N	19	1	0	0
		(%)	(95)	(5)		
4	20	N	20	0	0	0
		(%)	(100)			

Table 66: Mating performance and fertility

Mating performance and fertility - group values (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	20	20	20	100	100	100
2M	20	20	17	100	85	85
3M	20	20	20	100	100	100
4M	20	20	19	100	95	95
1F	20	20	20	100	100	100
2F	20	20	17	100	85	85
3F	20	20	20	100	100	100
4F	20	20	19	100	95	95

The sponsor calculated litter data based on 20, 17, 20, and 18 litters in the control, LD, MD, and HD groups. The sponsor reported no drug-related effects on F1 reproduction, although a slight reduction in the number of corpora lutea in the HDF was noted. The average numbers of corpora lutea were 17.0, 16.4, 16.8, and 15.7 in the control LD, MD, and HD groups (SDs were similar, 2.27 to 2.73). See sponsor's Table 67, below. (Note that data from the HDF with a single resorption were excluded, which yields reductions in a number of the fertility parameters

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for the HDF group. For reference, the reviewer-calculated means including that HDF are 15.0 for corpora lutea, 14.4 for implantations, 0.89 for early resorptions, 0.37 for late resorptions, 1.26 for total resorptions, 13.2 for live embryos, 7.0 for pre-implantation loss %, and 12.7 for post-implantation loss %.)

Table 67: Litter data

Litter data - group mean values on Day 14 of gestation (F1)

Group	:	1	2	3	4
Compound	:	Control	Tasimelteon	Tasimelteon	Tasimelteon
Dose (mg/kg/day)	:	0	50	150	450

Group /Sex		Corpora Lutea	Implantations	Resorptions Early	Resorptions Late	Resorptions Total	Live Embryos	Implantation Loss (%) Pre-	Implantation Loss (%) Post-
Statistical test:		Wi	Wi	Wc	Wc	Wc	Wi	Wa	Wa
1F	Mean	17.0	15.8	1.4	0.1	1.5	14.3	6.6	9.8
	SD	2.54	2.07				2.87		
	N	20	20	20	20	20	20	20	20
2F	Mean	16.4	15.2	1.4	0.0	1.4	13.8	8.5	11.0
	SD	2.27	2.67				3.73		
	N	17	17	17	17	17	17	17	17
3F	Mean	16.8	15.2	1.0	0.5	1.4	13.8	10.0	10.3
	SD	2.73	2.50				3.75		
	N	20	20	20	20	20	20	20	20
4F	Mean	15.7	15.2	0.9	0.4	1.3	13.9	4.6	7.9
	SD	2.42	2.18				1.84		
	N	18	18	18	18	18	18	18	18

Wa Treated groups compared to Control using Wald's test
Wc Treated groups compared to Control using Wilcoxon rank sum test
Wi Treated groups compared to Control using Williams' test.

Other (TK): Plasma exposures to tasimelteon (VEC-162) and metabolites M9, M12, and M14 were measured in the F1 offspring on LD 4 were as follows (see sponsor's Tables 73 to 76). Metabolite M13 plasma exposures were below the limit of quantitation, with the exception of males from one litter (~8 ng/mL, 4 hr post dose of dam). Some sex differences were observed (cf. tasimelteon C_{max} and AUC). The measured AUCs in the F1 pups at LD on LD4 exceed the AUC for a 20 mg dose in humans (411 ng·hr/mL).

Table 73 Pharmacokinetic parameters of tasimelteon in male and female F1 offspring on Day 4 of age following daily oral gavage administration of tasimelteon to female rats (F0) from Day 6 of gestation until Day 4 of lactation

Male offspring

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-8h} (ng.h/mL)	k (hours^{-1})	t½ (hours)
50	234	2	618	a	-
150	225	2	722	a	-
450	478	2	1830	a	-

Female offspring

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-8h} (ng.h/mL)	k (hours^{-1})	t½ (hours)
50	1230	2	2720	a	-
150	236	2	760	a	-
450	1260	2	3290	a	-

a Terminal rate constant could not be adequately estimated

Table 74 Pharmacokinetic parameters of M9 in male and female F1 offspring on Day 4 of age following daily oral gavage administration of tasimelteon to female rats (F0) from Day 6 of gestation until Day 4 of lactation

Male offspring

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-8h} (ng.h/mL)	k (hours^{-1})	t½ (hours)
50	300	2	1110	a	-
150	406	8	2160	a	-
450	474	8	2870	a	-

Female offspring

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-8h} (ng.h/mL)	k (hours^{-1})	t½ (hours)
50	361	2	1350	0.3216	2.2
150	412	4	2620	s	-
450	513	2	3090	a	-

a Terminal rate constant could not be adequately estimated

Table 75 Pharmacokinetic parameters of M12 in male and female F1 offspring on Day 4 of age following daily oral gavage administration of tasimelteon to female rats (F0) from Day 6 of gestation until Day 4 of lactation

Male offspring

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-8h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	208	8	865	a	-
150	358	8	1470	a	-
450	437	8	2290	a	-

Female offspring

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-8h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	156	8	762	a	-
150	389	8	1560	a	-
450	549	8	2450	a	-

a Terminal rate constant could not be adequately estimated

Table 76 Pharmacokinetic parameters of M14 in male and female F1 offspring on Day 4 of age following daily oral gavage administration of tasimelteon to female rats (F0) from Day 6 of gestation until Day 4 of lactation

Male offspring

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-8h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	-[a]	-[a]	-[a]	-[a]	-[a]
150	8.00	8	-[b]	c	-
450	14.1	8	79.1	c	-

Female offspring

Dose level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-8h} (ng.h/mL)	k ($hours^{-1}$)	t½ (hours)
50	-[a]	-[a]	-[a]	-[a]	-[a]
150	9.72	8	-[b]	c	-
450	17.1	8	90.1	c	-

[a] Mean plasma concentrations were BLQ at all sampling times
[b] Mean concentrations were only quantifiable at one sampling time; AUC_{0-8h} not reported
c Terminal rate constant could not be adequately estimated

10 Special Toxicology Studies

The sponsor conducted ocular irritancy and skin sensitization tests. Also, a photo safety assessment was undertaken; a tissue distribution result suggested that tasimelteon binds melanin.

Study 99AB38.350: BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY
Conducted by [redacted] (b) (4) Dated 4/22/99
GLP (except test article characterization)
Drug: Tasimelteon (BMS 214778-01), COA not provided

Tasimelteon scored an average *in vitro* score of 1.5 (mild irritant) on the Bovine Corneal Opacity and Permeability Assay (BCOP). Five freshly obtained corneas were tested. Saline (0.9% USP injectable saline [(b) (4) was added to solid BMS 214778-01 to achieve a 20% mixture (w/v) and the vial was vortexed for approximately one minute. A 20% (w/v) solution of imidazole was used as the positive control. See the sponsor's excerpted explanation of scores and summary Table 1 of results, following. Of the five individual corneas, the *in vitro* score ranged from 0.3 to 5.3 (all mild irritant).

The following classification system was established by Sina et al.[1] based on studies with a wide range of test materials. While this classification system provides a good initial guide to interpretation of these *in vitro* data, these specific ranges may not be applicable to all classes of materials.

in vitro score:

from 0 to 25	= mild irritant
from 25.1 to 55	= moderate irritant
from 55.1 and above	= severe irritant

Table 1

IIVS Test Article Number	Sponsor's Designation	Opacity Measurement	Permeability Measurement	*In Vitro* Score	pH of dosing solution
99AB38	BMS 214778-01	1.7	-0.013	1.5	6.0
Positive Control	Imidazole	62.8	1.588	86.7	NA

NA - Not Applicable

Study BMY 378/993273/SS: BMS 214778-01 SKIN SENSITIZATION TO THE GUINEA-PIG (MAGNUSSON & KLIGMAN METHOD)
Conducted by (b) (4) Dated 8/20/01
GLP, except for test article characterization
Drug: Tasimelteon (BMS 214778-01), lot 032, purity 99.3%

Ten healthy female guinea pigs (nulliparous, nonpregnant; 4-7 weeks of age; 349-400 g) were tested with tasimelteon; 5 animals were used as controls. Intradermal and

Reference ID: 3399188

topical irritancy was tested at the following concentration (see the sponsor's summary, below).

Selection of concentrations of test substance for the main study

Based on the results of the preliminary investigations, the following concentrations of BMS 214778-01 were selected:

Induction intradermal injection - 2.5% w/v in Alembicol D

This was the maximum practical concentration for intradermal administration and caused irritation but did not adversely affect the animals.

Induction topical application - 50% w/v in Alembicol D

This was the maximum practical concentration that could be prepared and dosed topically and produced some irritation but did not adversely affect the animals.

Topical challenge - 30 and 15% w/v in Alembicol D

From preliminary investigations 30% w/v in Alembicol D was the highest concentration not giving rise to irritating effects.

Following initial exposure to tasimelteon (the 'induction' period comprising intradermal injections and topical application) the animals were subjected, approximately two weeks after the topical induction exposure, to a 'challenge' exposure of the test substance in order to establish if a hypersensitive state had been induced. During preliminary investigations, the "maximum practical concentration" of tasimelteon for topical application (50%) produced slight irritation in 2 of 4 animals. Dermal responses to the induction and challenge phases were scored as follows (see excerpt from the sponsor's submission). Sponsor's Tables 1, 2, and 3 below provide the results of the induction and two challenge phases. In this study tasimelteon did not produce evidence of skin sensitization (delayed contact hypersensitivity) in any of the ten test animals.

Dermal responses

The dermal reactions resulting from intradermal injection and topical application on the preliminary study, and topical application at the challenge were assessed using the following numerical system:

Erythema and eschar formation:

No erythema	0
Slight erythema	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation:

No oedema	0
Slight oedema	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

TABLE 1

Dermal reactions observed after each induction

Group	Animal number	Intradermal injections Site number 1	2	3	Topical application
Control	1595	N	1	N	0
	1596	N	1	N	0
	1597	N	1	N	0
	1598	N	1	N	0
	1599	N	1	N	0
Test	1600	N	1	N	0
	1601	N	1	N	0
	1602	N	1	N	0
	1603	N	1	N	0
	1604	N	1	N	0
	1605	N	1	N	0
	1606	N	1	N	0
	1607	N	1	N	0
	1608	N	1	N	0
	1609	N	1	N	0

Intradermal injections

Control animals: See figure 1 (previous page)
Test animals: See figure 1 (previous page)

N Necrosis
0 No irritation
1 Slight irritation

Topical application

Control animals: Alembicol D
Test animals: BMS 214778-01, 50% w/v in Alembicol D

0 No erythema

TABLE 2

Dermal reactions observed after the first challenge application with BMS 214778-01

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score 24 Hours A	24 Hours P	48 Hours A	48 Hours P
1595	E	1	1	1	0
	O	1	0	0	0
1596	E	2	1	2	0
	O	1	0	1	0
1597	E	1	0	1	0
	O	1	0	0	0
1598	E	1	0	1	0
	O	0	0	0	0
1599	E	1	0	0	0
	O	0	0	0	0

A Anterior site, exposed to BMS 214778-01, 30% w/v in Alembicol D
P Posterior site, exposed to BMS 214778-01, 15% w/v in Alembicol D

TABLE 2

Dermal reactions observed after the first challenge application with BMS 214778-01 (continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score			
		24 Hours		48 Hours	
		A	P	A	P
1600	E	1	0	0	0
	O	0	0	0	0
1601	E	1	0	1	0
	O	1	0	0	0
1602	E	1	L1	1	0
	O	1	0	0	0
1603	E	1	0	1	0
	O	1	0	0	0
1604	E	2	1	1*	0
	O	1	0	0	0
1605	E	0	0	0	0
	O	0	0	0	0
1606	E	1	0	0	0
	O	0	0	0	0
1607	E	0	0	0	0
	O	0	0	0	0
1608	E	1	0	0	0
	O	0	0	0	0
1609	E	2	1	1	0
	O	1	0	0	0

L Localised dermal reaction (restricted to a small area of the challenge site)
* Dryness and sloughing of the epidermis
A Anterior site, exposed to BMS 214778-01, 30% w/v in Alembicol D
P Posterior site, exposed to BMS 214778-01, 15% w/v in Alembicol D

TABLE 3

Dermal reactions observed after the second challenge application with BMS 214778-01

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score			
		24 Hours		48 Hours	
		A	P	A	P
1595	E	0	0	0	0
	O	0	0	0	0
1596	E	0	0	0	0
	O	0	0	0	0
1597	E	0	0	0	0
	O	0	0	0	0
1598	E	0	0	0	0
	O	0	0	0	0
1599	E	0	0	0	0
	O	0	0	0	0

A Anterior site, exposed to BMS 214778-01, 15% w/v in Alembicol D
P Posterior site, exposed to BMS 214778-01, 7.5% w/v in Alembicol D

TABLE 3

Dermal reactions observed after the second challenge application with BMS 214778-01 (continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score				Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		
		A	P	A	P	
1600	E	0	0	0	0	-
	O	0	0	0	0	
1601	E	0	0	0	0	-
	O	0	0	0	0	
1602	E	0	0	0	0	-
	O	0	0	0	0	
1603	E	0	0	0	0	-
	O	0	0	0	0	
1604	E	0	0	0	0	-
	O	0	0	0	0	
1605	E	0	0	0	0	-
	O	0	0	0	0	
1606	E	0	0	0	0	-
	O	0	0	0	0	
1607	E	0	0	0	0	-
	O	0	0	0	0	
1608	E	0	0	0	0	-
	O	0	0	0	0	
1609	E	0	0	0	0	-
	O	0	0	0	0	

A Anterior site, exposed to BMS 214778-01, 15% w/v in Alembicol D
P Posterior site, exposed to BMS 214778-01, 7.5% w/v in Alembicol D

Study No. VCR-TMI-121012: MOLAR EXTINCTION COEFFICIENT DETERMINATION FOR TASIMELTEON AND TASIMELTEON METABOLITES M3, M9, M11, M12, M13, and M14

Conducted by [redacted] (b) (4) but reported by Vanda
Non-GLP report dated 1/29/13

A Molar Extinction Coefficient (MEC) determination was performed for tasimelteon and its metabolites. The solutions for all compounds except M11 were prepared at a concentration of 0.03 mg/mL in deionized water; due to better solubility, the solution for M11 was prepared at a concentration of 0.1 mg/mL. All UV determinations were performed with a cell path length of 1 cm. Because the absorption maxima for tasimelteon and all metabolites occurred below 290 nm, which is the lower limit of the 290 nm to 700 nm wavelength range specified in the ICH guidance, the MEC values were calculated at both the absorption maxima and at 290 nm. There was no absorption in the 300 nm to 700 nm wavelength range for tasimelteon or its metabolites. The obtained MEC values for tasimelteon and its metabolites M3, M9, M11, M12, M13, and M14 are provided in sponsor's Table 1, below.

Table 1: MEC values for tasimelteon and metabolites

Compound	MEC (L/mol*cm)
Tasimelteon	ε (283nm) = 1748.84
	ε (290nm) = 689.41
Metabolite M3	ε (285nm) = 1815.38
	ε (290nm) = 1125.42
Metabolite M9	ε (276nm) = 1558.91
	ε (289nm)* = 76.69
Metabolite M11	ε (275nm) = 1556.93
	ε (290nm) = 127.51
Metabolite M12	ε (285nm) = 2968.42
	ε (290nm) = 2021.44
Metabolite M13	ε (275nm) = 1513.57
	ε (290nm) = 97.97
Metabolite M14	ε (285nm) = 2702.42
	ε (290nm) = 1798.80

*MEC at 290 nm was zero, so MEC at 289 nm is reported

11 Integrated Summary and Safety Evaluation

Pharmacology

Tasimelteon and a number of its metabolites showed affinity at melatonin (MT_1 and/or MT_2) receptors, with slightly greater affinity at MT_2 receptors. Tasimelteon and metabolite M13 showed nanomolar affinity at MT_1 receptors; tasimelteon, M11, M12, M13, and M14 showed nanomolar affinity at MT_2 receptors. In an early study, tasimelteon was shown to have affinity similar to melatonin at MT_1 receptors, and greater than melatonin at MT_2 receptors. Tasimelteon did not show affinity at other targets in a receptor binding screen.

Toxicology

With regard to this application, there are three general issues that warrant discussion as they potentially impact the adequacy of the toxicology development program: the use of PEG-400 as a vehicle for the drug, the termination (and subsequent reopening) of a number of the toxicology studies, and coverage of the exposures to the major human circulating metabolites (M9, M12, and M13) in the toxicological species.

For the toxicity studies PEG-400 has been used as the vehicle. Although relatively widely used as an excipient, both toxic and some protective effects have been observed for PEG-400 itself. According to Dr. Atrakchi's review, the sponsor indicated that the high doses tested were limited by the toxicity of PEG-400 itself and the finding of cecal dilation in the 2-week rat toxicity study was attributed to PEG-400. GI and related effects (e.g., loose feces, reduced food consumption, reduced body weights) have been observed with PEG-400 (Hermansky et al., 1995), and renal toxicity has been suggested. More recent evidence demonstrated that when administered by gavage (5, 50, or 100% v/v% at 5 ml/kg/day), PEG-400 caused histopathological changes localized

to the stomach mucosa, but not in the intestine (Ueda et al., 2011). Conversely, it has been shown that PEG-400 protects against some compounds causing gastric mucosal damage (cf. Gutierrez-Cabano, 1995, Gutierrez-Cabano, 2000). It is unclear whether the use of PEG-400 as the vehicle had an effect on the apparent toxicity profile of tasimelteon. While body weights and food consumption were affected in a number of studies, there were no clearly adverse histopathological effects on stomach and/or forestomach.

Secondly, it is notable that the chronic toxicity assays in rat and monkey were terminated after the in-life portion of the assays during development and summary and/or interim reports were issued; upon restarting development, the sponsor contracted CROs to complete the histopathology assessments for these studies. (The rat fertility study was also completed in this manner.) However, GLP and QA statements have been submitted for these studies (although a separate, signed pathology report for the chronic, 6-month rat toxicity study was not included in the report and was submitted in a later NDA submission only upon request). Although it is an unusual circumstance, these studies appear to have been completed in a reasonably appropriate manner.

According to the Clinical Biopharmaceutics staff, M9, M12, and M13 were identified as the major human circulating metabolites, with exposures 130%, 180%, and approximately equal to those of tasimelteon, respectively. With the exception of the pre-/post-natal development study in rats, exposures to the major human metabolites were not measured in the toxicology studies. The sponsor conducted a separate study to determine exposures to the major human metabolites at steady state in rats, and used the obtained ratios (metabolite/tasimelteon) to extrapolate the metabolite exposures achieved in the toxicology studies. Only single dose metabolite exposure data were obtained for monkey, and only exposure data for M11 (not a major human circulating metabolite) were obtained at steady state in mice and at the HD tested in the embryofetal study in rabbits. Estimated exposures to M9, M12, and M13 at the high doses in the rat toxicology studies, using the steady state metabolite exposure ratios obtained, suggest that the major human circulating metabolites have been tested at exposures equal to or exceeding those anticipated in humans at the RHD. The one exception is offspring exposure (measured on LD4) to metabolite M13. Metabolite coverage was not always achieved at the observed NOAEL doses.

General Toxicity

The chronic toxicity studies generally reflected the toxicities demonstrated in subchronic studies, including slight effects on body weight and food consumption, clinical pathology, and a number of target organs (i.e., liver, kidney, and reproductive organs, as well as spleen and endocrine organs). Liver toxicity, in particular, was of increased severity and demonstrated at lower doses. Following the summaries of the chronic rat and monkey studies, an overview of the target organs/systems of tasimelteon toxicity will be discussed.

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The 6-month chronic toxicity study in rats tested doses of 0, 5, 50, and 500 mg/kg. Convulsions (tonic and/or clonic), tremors, hypoactivity, labored respiration, ataxia, loss of righting reflex were observed in rats. In rats, pica was observed in both sexes at HD, and in females at MD. Over the course of the study, average body weights were slightly reduced in males, but a similar effect was not seen in females. Several clinical pathology changes were observed. Slight reductions in RBC parameters were observed in females, and reticulocytes and/or platelets were slightly increased in both sexes at HD. WBC increases were also observed in both sexes (especially, neutrophils, monocytes [F], LUC [F], and basophils [M]). PT time and fibrinogen showed slight increases. ALT, cholesterol, and triglycerides were increased in HDF (some increase in males); BUN and creatinine were slightly reduced. Serum electrolytes showed very slight but consistent changes over the treatment period (sodium and chloride slightly reduced, calcium slightly increased). Urine volume was increased at HD. Grossly, enlarged liver was observed in all HD animals. There was an increased incidence of dilatation of the uterus in treated females. Liver, heart, adrenal gland, kidney, ovary (F), and spleen (F) weights were increased; pituitary gland and prostate (M) weights were reduced. Histologically, liver, kidney, spleen, and endocrine/reproductive organs were identified as target organs. In the liver, minimal to moderate centrilobular hepatocyte hypertrophy (≥MD) and minimal to slight focal hepatocyte necrosis (4 HDM and 3 HDF) were observed. In the kidney, exacerbated (slight to moderate) chronic progressive nephropathy, minimal to slight cortical tubules with hyaline droplets, minimal to slight cortical tubular pigment, and interstitial pigmented macrophages were observed in both sexes in a roughly dose-related manner. In MD and/or HD females, slight cortical tubular dilation, interstitial inflammation, and simple tubular hyperplasia were also observed. In the spleen, minimal to slight hemosiderosis and minimal to slight increased extramedullary hematopoiesis were observed. The sponsor considered this reflective of an increased rate of RBC removal from peripheral blood leading to the "compensatory" response. Thyroid changes (called prominent ultimobranchial cysts by the pathologist) were observed in 3 HDM and 7 HDF (compared to 1 Con/ sex); focal pituitary hyperplasia of the pars distalis was also observed at HD (one/sex; see Zabka et al., 2011). The incidence of ovarian follicular cysts (4 HDF vs. 1 ConF) and luminal dilatation of the uterus (14 HDF vs. 6 ConF) were increased at HD. In the mammary gland, two HDF showed galactocele(s) and three HDF showed secretory activity. Plasma tasimelteon exposures were greater in females, some accumulation at LD, and slightly reduced exposures with repeated dosing for 6 months (at ≥MD in males, HD in females).

The 1-year toxicity in monkey tested 0, 3, 20, and 150 mg/kg. Convulsions (2HD), flaccidity (HDM with convulsion), labored breathing, decreased activity (HD), salivation (≥ MD), emesis (F, ≥ MD), alopecia (≥ MD), and decreased appetite were observed. The sponsor noted that for a single two-day period, one LDM was noted to be coldness, prostrate, ataxic, weak, unable to sit up, slightly dehydrated, and had pale mucous membranes; this animal showed elevated LFTs and decreased wbc and RBC counts. Slightly reduced average body weights were observed at HD over the first 6 months of the study (which appeared to resolve), and slightly increased average body weights were observed over the last few months of the study at LD. Blood pressure was slightly

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increased in HDF on D269 and D365. Reductions in RBC parameters (<15%) were observed at HD, and increased platelets and decreased fibrinogen were suggested. Serum ALT (2-3x) and GGT (50-70%) were increased at HD, and ALP was decreased (30-40%); triglycerides were increased (~2x) in HDM. Liver weights were increased (1.4-1.8x) at HD, as was thyroid weight (~40%) in MD and HD males. Few clear histologic changes were observed. Although noted only in the comment field but discussed by the sponsor, the three remaining HDF at terminal sacrifice showed a lack of corpora lutea; the sponsor indicated that this could indicate a "lack of recent estrus cycling." Mild focal necrosis, minimal to mild periportal fibrosis, mild focal serosal fibrosis, mild subserosal fibrosis, centrilobular fatty change, and/or minimal focal pigmented macrophages were observed in the livers of individual MDM, MDF, HDF and HDM. The incidence of minimal to mild chronic interstitial inflammation in the kidney was increased in treated males. Minimal chronic inflammation of the heart and/or aorta was observed in a few treated animals. Plasma tasimelteon exposures showed some reduction with repeated dosing for a year.

Target Organs/Systems

The primary target organs of tasimelteon toxicity include: the CNS, liver, kidney, and reproductive organs. Some alterations were also observed in the hematologic and endocrine systems (i.e., thyroid, pituitary, and adrenal glands), and related organs.

Generally slight reductions in weight and sometimes food consumption were observed across the studies; these were usually not severe enough to hamper the studies significantly. Post-dosing emesis was observed in monkeys, and salivation was observed in many species. In the chronic study in rats, pica was observed in both sexes at HD, and in females at MD; average body weights were slightly reduced in males, but a similar effect was not seen in females. In the one-year monkey study, slightly reduced average body weights were observed at HD over the first 6 months of the study (which appeared to resolve), but slightly increased average body weights were observed over the last few months of the study at LD.

CNS

CNS signs including convulsions (clonic and/or tonic), tremors, loss of righting reflex, flaccidity, and/or ataxia were observed in single dose studies using higher doses and usually at relatively high doses tested for longer durations in rats, mice, and monkeys. Reduced activity, ataxia, and occasionally labored breathing were noted throughout many studies. Of unclear relationship to drug were observations in one LDM (3 mg/kg) in the 12-month monkey study; this animal was noted to be cold, prostrate, ataxic, weak, unable to sit up, and slightly dehydrated, and had pale mucous membranes, for a single two-day period; this animal showed elevated LFTs and decreased WBC and RBC counts. A few of these signs resembled those seen at higher doses (and could therefore be considered drug-related), but the overall severity of the findings were not replicated in a clearly dose-related manner in animals treated with higher doses. If the signs in this animal were, in fact, drug-related, it appears to be an outlier.

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Liver

The liver was clearly a target organ in most of the toxicological species used. Rodents showed the effects on liver most clearly. Subchronic studies in rats showed increased liver weight and size, dose-dependent minimal to marked centrilobular to panlobular hepatocellular hypertrophy (generally at ≥ 100 mg/kg), and increased hepatocellular mitoses at high dose (400 mg/kg). In the chronic (6-month) study, minimal to slight focal hepatocyte necrosis (500 mg/kg, males and females) was also observed; tumors were observed in the rat carcinogenicity study at ≥ 100 mg/kg. These histopathologic changes were accompanied by clinical pathology changes such as increased: ALT, globulin, albumin, total protein, cholesterol, glucose (F), and/or triglycerides (F); these were generally at least partially reversible in subchronic studies. The NOAEL in rats generally decreased with increasing dose (e.g., 50 mg/kg for 1-week, 25 mg/kg for 1-month, 5 mg/kg for 6-months). Increased liver weights, dose-related minimal to moderate centrilobular hepatocellular hypertrophy, and slightly increased serum albumin and total protein (M) were also observed in mice. A number of the effects, particularly in rats, may be related to hepatic enzyme induction (demonstrated in a separate study using liver tissue samples from TK animals at 6 months in the rat carcinogenicity bioassay), but there is some evidence of damage in a few animals at higher doses (i.e., necrosis, regeneration, fibrosis).

In monkeys, the effects in liver were more pronounced (and at lower doses) with longer duration of treatment. In a 1-week study, mild changes in clinical pathology parameters (including increased serum triglycerides (M), glucose (F), ALT (≥100 mg/kg), and fibrinogen, and decreased sodium (M), chloride, and phosphorus) were observed after 1-week (175 mg/kg). After a month of treatment, 125 mg/kg resulted in increased liver weights (≥ 45 mg/kg); the reported no-effect dose was 15 mg/kg. After 1-year of treatment, histopathological changes were observed in individuals (at ≥ 20 mg/kg; including mild focal necrosis, minimal to mild periportal fibrosis, mild focal serosal fibrosis, mild subserosal fibrosis, centrilobular fatty change, and/or minimal focal pigmented macrophages), liver weights were increased (at 150 mg/kg; 1.4-1.8x), and clinical pathology changes were observed (at 150 mg/kg; increased serum ALT (2-3x), GGT (50-70%), and triglycerides were increased (~2x in M)).

Kidney

Kidney toxicity was most clearly observed in rats (that demonstrate species-specific renal toxicity with aging, chronic progressive nephropathy [CPN]); however, the pattern did not strictly follow the usual pattern for CPN and some evidence was observed in other species. Kidney toxicity was observed with as little as 2-weeks of tasimelteon administration in males rats, demonstrated as hyaline droplet nephropathy. In a 1-month study in rats, increased kidney weight (only 400 mg/kg) and dose-dependent hyaline droplet nephropathy (≥ 100 mg/kg) were observed in males; however, decreased serum urea nitrogen and creatinine were observed. In the 6-month study in rats, both sexes showed

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histopathological alterations with other concomitant alterations. At HD, BUN and serum creatinine continued to be slightly reduced, and serum electrolytes showed very slight but consistent changes over the treatment period (i.e., sodium and chloride slightly reduced, calcium slightly increased). Kidney weight was increased, as was urine volume. Histologically, exacerbated (slight to moderate) chronic progressive nephropathy, minimal to slight cortical tubules with hyaline droplets, minimal to slight cortical tubular pigment, and interstitial pigmented macrophages were observed in both sexes in a roughly dose-related manner. In MD and/or HD females, slight cortical tubular dilation, interstitial inflammation, and simple tubular hyperplasia were also observed. In this study, there was a suggestion of equal or slightly greater severity findings in females, which does not follow the generally male-predominant pattern accepted for CPN. In mice, kidney weights were increased in males at high doses (i.e., 600 mg/kg) in the 3-month study, but were slightly reduced in the carcinogenicity bioassay and showed minimal histopathological changes (e.g., few animals with cortical tubules with hyaline droplets and/or pigment). Monkeys showed minimal evidence of renal toxicity (decreased sodium, chloride and phosphorus in the 1-week study; minimal to mild chronic interstitial inflammation and medullary tubule cyst in treated males in the 1-year study).

Reproductive organs

A number of reproductive organ effects occurred in rodents, rabbits, and monkeys. In female rats, several effects were seen in ovary (i.e., increased ovary weight, of ovarian follicular cysts, absent corpora lutea, and interstitial or Sertoli cell hyperplasia),uterus (i.e., dilatation, luminal dilatation, glandular dilatation, glandular hyperplasia, adenocarcinomas, and squamous cell carcinomas), and uterine cervix (i.e., epithelial hyperplasia and keratinization, mural/stromal hypertrophy/hyperplasia, squamous cell carcinomas). In the mammary gland, galactocele(s), secretory activity, ductular dilatation, and a slightly increased incidence [nss] of adenocarcinoma (at 250 mg/kg) were observed. In male rats, effects were observed in the prostate gland (i.e., decreased weight, inflammation) and testis (i.e., interstitial cell hyperplasia and a slight increase in Leydig cell adenomas in the carcinogenicity bioassay). Mice showed fewer, but similar, effects, including: cysts in the ovary and uterus, decreased prostate weight (600 mg/kg), epithelial hyperplasia in the seminal vesicles, and cystic atrophy of the preputial gland. In the EFD study, rabbits demonstrated small ovary and black ovarian cysts. A few changes were also observed in monkey, including: decreased seminal vesicle and prostate and testes weights (1 –month), lactation of the mammary gland (1 HDF in the 1-year study), and a lack of corpora lutea in the ovary (HDF at terminal sacrifice in the 1-year study); the sponsor indicated that the last finding could indicate a "lack of recent estrus cycling," which is consistent with the irregular estrus cycles demonstrated in rats.

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Hematologic System (Including Spleen)
Effects on hematologic function were observed, mostly at high doses, in rodents and monkeys. Generally slight to mild (5 to 15%) reductions in red blood cell parameters were observed, with increases in reticulocytes and/or platelets. In an early (1-month) subchronic rat study, RBC polychromasia and anisocytosis were observed at 400 mg/kg. In the chronic rat study, slight increases in WBC were also observed in both sexes. Effects in the spleen (rat carcinogenicity and/or 6-month rat study) reflected these alterations (i.e., increased spleen weight (F), minimal to slight hemosiderosis (M and F), and/or minimal to slight increased extramedullary hematopoiesis); the sponsor considered this reflective of an increased rate of RBC removal from peripheral blood leading to the "compensatory" response.

Endocrine System
Alterations in the endocrine system (i.e., adrenal, thyroid, and pituitary) were suggested. In rat, increased adrenal weights were observed but did not show clear histopathological changes; however, in the rat carcinogenicity bioassay, VEC-162-treated animals showed cortical/cystic/hemorrhagic degeneration and/or sinusoidal dilatation/congestion. In the In the 6-month study in rats, alterations were observed in endocrine organs at HD, but generally with low incidence: decreased pituitary weight, cysts and/or focal hyperplasia (and/or hypertrophy) of the pars distalis of the pituitary (6-month study, carcinogenicity bioassay), and thyroid changes (prominent ultimobranchial cysts in the 6-month study, hypertrophy, atrophy, and/or cyst in the carcinogenicity bioassay). Some of these findings may reflect the observed hepatic enzyme induction in rats (cf. Zabka et al., 2011), or may be a reflection of aging. In the 1-yr monkey study, increased thyroid weight (~40%) was observed males (≥ 20 mg/kg; but was slightly, ~20%, decreased in females); histologically, only a few instances of follicular cysts were observed in treated monkeys (not clearly dose-related).

Heart
Minimal evidence of alterations in heart was observed; primarily these were seen as changes in organ weight. Increased heart weights were observed in female rats, without clear histologic changes. However, heart weight was slightly reduced carcinogenicity bioassay in mice. In monkeys, minimal chronic inflammation of the heart and/or aorta was observed in a few treated animals; slightly increased blood pressure was observed in HDF late in the 1-year study.

Genetic Toxicology
Tasimelteon was neither mutagenic in an Ames assay nor clastogenic in an *in vivo* rat micronucleus assay (see review by Dr. Atrakchi, dated 1/16/98); however, an *in vitro* chromosomal aberration assay demonstrated clastogenic effects. It is noted that the rat micronucleus assay was conducted as part of the 1-month toxicity study with tasimelteon and had some flaws (i.e., it did not use a positive control and there is some question about the adequacy of the maximum dose tested). The sponsor also investigated the genotoxicity of M11 (a non-major human circulating metabolite that has

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poor coverage in the animal species). Metabolite M11 was not mutagenic, but was clastogenic in an *in vitro* chromosomal aberration assay in CHO cells. Taken together, there is limited evidence that tasimelteon has genotoxic properties. It is notable that a similar compound, ramelteon, was also shown to be positive in a chromosomal aberration assay. Given the positive results in the carcinogenicity assay in rats, there would not seem to be a need to conduct additional genetic toxicology studies.

Carcinogenicity

Two-year carcinogenicity bioassays were conducted in rats and mice.

Tasimelteon was administered orally (by gavage) at doses of 0 (vehicle: PEG-400), 20 (LD), 100 (MD), and 250 (HD) mg/kg male and female Sprague Dawley rats (65/sex/group) for up to 104 weeks. Dosing was suspended for HDF during week 101/102 when the number of survivors fell to 20, and this group was terminated at 104 weeks. All male groups were terminated at 103 weeks due to low survival in the male control group. Overall, there was no statistically significant drug-related effect on survival rates in males or females. Body weights at 102 weeks were 96%, 96%, and 93% those of control males in the LD, MD and HD groups, respectively. In females, body weights at 104 weeks were 108%, 97%, and 95% those of controls in the LD, MD, and HD groups, respectively; however, during weeks 76-88, body weight in HDF was approximately 87% that of control females. The sponsor indicated that tumors were observed in liver (MD & HD, both sexes), uterus, and uterine cervix. In addition to these tumors, there were non-significant increases in neoplasms in ovary, mammary gland, testes, and skin. FDA biostatistical evaluation indicated that only the incidence of uterine endometrial adenocarcinomas in HDF reached statistical significance. The FDA Executive CAC concluded that the observed neoplasms in liver, uterus and cervix were drug- related, based on statistical significance or exceeding the historical control (HC) range (see table below; cf. meeting minutes dated 10/16/13). Histopathologic alterations were observed in liver (e.g., centrilobular hepatocyte hypertrophy, centrilobular hepatocyte vacuolation, cystic degeneration, regenerative and bile duct hyperplasia, pigment in hepatocytes), kidney (e.g., cortical tubular pigment, tubular basophilia or hypertrophy, pelvic dilatation), ovary (e.g., cysts), uterus (glandular dilatation and hyperplasia), uterine cervix (epithelial hyperplasia and keratinization), mammary gland (duct dilatation, galactoceles), testes (interstitial cell hyperplasia), and pituitary (e.g., focal pars distalis hyperplasia and hypertrophy).

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Organ/Tumor	Sex	Incidences				p values				HC (Range)
		Con	LD	MD	HD	Trend	LD vs C	MD vs C	HD vs C	
Uterus, Endometrial Adenocarcinoma	F	2	2	2	11	0.0002	0.6360	0.6360	0.0035	0
Liver, Adenoma	F	2	1	6	7	0.0087	0.8511	0.0980	0.0539	0 – 3
Liver, Adenoma + Carcinoma	M	2	2	8	8	0.0145	0.6222	0.0746	0.0444	2 – 4
Uterus + Cervix, Squamous Cell Carcinoma	F	1	0	1	5	0.0059	1	0.7160	0.0765	0

Tasimelteon was administered orally (by gavage) at doses of 0 (vehicle: PEG-400), 30 (LD), 100 (LMD), and 300 (HD) mg/kg male and female CD-1 mice (66/sex/group) for up to 104 weeks. There was no drug-related effect on survival rates in males or females. At week 104, body weight in HDM was reduced approximately 10% compared to control males. Body weight was not affected in females. No drug-related neoplasms were identified. Slight, but non-significant, increases were observed in intestines (male) and uterine cervix. Few histopathologic changes were observed in the study, most notably centrilobular hypertrophy in the liver.

The sponsor postulated mechanisms underlying the observed increases in liver, uterine and uterine cervix tumors in rats that they believe to negate, or ameliorate, human relevance. The sponsor attributed the observed hepatic tumors to hepatic enzyme induction in rats. While there is some suggestive evidence (e.g., enzyme induction, hepatic hypertrophy with early evidence of increased hepatocellular mitoses), it is not clear whether the increased liver tumor incidences in rats is related to the observed hepatic enzyme induction (cf. Ennulat et al., 2010; Holsapple et al., 2006; IARC Monograph 79, 2002; Maronpot et al., 2010; Whysner et al., 1996; Williams et al.,2001; Williams, 1997; Wojcikowski & Daniel, 2009), particularly since some evidence of other potential mechanisms was observed (i.e., damage, clastogenicity). Additionally, it is notable that clear effects on thyroid were not observed. (As a side note, the observed increases in uterine and uterine cervix tumors may suggest an increase in exposures to estrogens, which is in itself a potential mechanism for hepatocarcinogenesis.) A marketed drug with a similar mechanism, ramelteon (Rozerem) also demonstrated increased hepatic tumors (although of greater incidence, and in both rats and mice) and clastogenicity; these effects were reported in the label and human relevance was not excluded. Without more objective and complete data, it is not clear that the relevance of the hepatic tumors to humans can be excluded.

With regard to the reproductive organ tumors, the sponsor attributed the tumors in the uterus and uterine cervix (i.e., endometrial adenocarcinoma and squamous cell carcinoma) to reduced serum prolactin levels, possibly resulting from chronic activation

of melatonin receptors in the pars tuberalis (cf. Morgan and Williams, 1996). However, no data were provided to support this hypothesis. The sponsor also stated that similar effects were not observed in mice, so the effects may be due to a "species-specific effect of unknown aetiology on aging female rats." Melatonin, and therefore melatonin receptors, are known to have roles in the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes (Chuff et al., 2011a; Chuff et al., 2011b; Diaz et al., 2000; Dubocovich & Markowska, 2005; Malpaux, 1999; Preslock, 1984; Slominski et al., 2012); these role(s) have not been fully elucidated. In general, melatonin is generally believed to result in a progesterone dominance over estrogens, and has been suggested to possess anticancer effects in estrogen-dependent cancers, especially breast cancer (cf., Cos et al., 2008; Dubocovich & Markowska, 2005). This does not appear to be the case with tasimelteon; although uterine endometrial tumors are generally believed to be an estrogen-dependent tumor (cf., Ito et al., 2007; Ito et al., 2011; Slominski et al., 2012), uterus and uterine cervix showed increased tumor incidences in rats. Additionally, and contrary to the sponsor's hypothesis, mammary gland did not appear protected from carcinogenesis (as would be expected with a drug that reduced serum prolactin); in fact, a slight, but non-significant, increase in adenocarcinomas was observed at HD. In males, only a slight, non-significant increase in Leydig cell hyperplasia and adenomas was observed in testes following administration of tasimelteon. Notably, the label for marketed drug ramelteon (Rozerem) does not indicate increases in female reproductive organ or mammary gland tumors, but does indicate an increase in Leydig cell tumors of the testes; increased prolactin and decreased testosterone have been reported in humans (see Richardson et al., 2009; Richardson & Wang-Weigand, 2009). There is not sufficient objective evidence to exclude human relevance of the uterine and uterine cervix tumors.

Reproductive Toxicology

The reproductive effects of tasimelteon were tested in rats and rabbits. As previously suggested in the toxicity studies (e.g., effects on reproductive organs, such as the lack of corpora lutea in the ovaries of female monkeys in the chronic toxicity study), effects on female fertility and fetal development (into adulthood) were demonstrated. A slight increase in infertile pairings demonstrated in a fertility study dosing both males and females later clarified that the effect on fertility resulted from tasimelteon exposures in the females. Irregular estrus cycles, slightly increased infertile pairings, and slight reduction sin fertility parameters were observed at MD and HD in females. The embryofetal development (EFD) studies in rats and rabbits demonstrated some embryofetal toxicity. Drug-related slight delays in development and decreased fetal body weights were observed at MD and HD in rats and rabbits. Rabbit does also demonstrated an increase in abortions at HD, and slightly increased late resorptions at MD and HD (although early resorptions were increased at LD and MD, which puts the significance of this apparent change in question). Some maternal toxicity was observed at HD (i.e., clinical signs, and slight reductions in body weight and food consumption) in the dams and does.

Clear toxicity in the F1 generation was demonstrated in the pre/post-natal study, particularly as developmental delays and a reduced growth rate postpartum and into

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adulthood. Limited F_0 maternal toxicity was demonstrated (i.e., slightly [~3-5%] reduced body weights during gestation at MD and/or HD, and during lactation in all VEC-162-treated dams). The gestation period was very slightly increased in all VEC-162-treated dams, and the live birth index was slightly reduced at HD. Pups from VEC-162-treated dams showed an increased incidence of "underactive," "swollen area on the abdomen,"and "small build." Although average pup birth weights were similar (<5% difference; provided as the average of the litter averages for each sex), HD pups demonstrated reduced (~15%) body weights throughout lactation and into adulthood. Sexual maturation was achieved at a lower body weight in males (similar PND) but was delayed in females. Other developmental milestones (e.g., righting responses) were similarly delayed. Learning and memory testing (Morris Water maze) demonstrated clear deficits in males (i.e., trial times, sector entries, failed trials), but not females, when tested at PND 31/32; when re-tested at PND 47/49, no consistent differences were reported. A few effects were demonstrated on F_1 generation reproduction. Reductions in body weight were accentuated in the adult female F_1 generation during gestation, and there was some evidence for reductions in fertility parameters (e.g., corpora lutea, implantations, late resorptions, post-implantation loss %, and live births) at HD. The F_1 generation was exposed to tasimelteon, M9, M12 and M14 (as measured on LD4), although to a much lesser extent than the dams.

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12 Appendix/Attachments

Appendix 1: Literature References Cited

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Appendix 2: Histopathology Inventory

Study	Rat	Monkey	2-yr Bioassay	2-Yr Bioassay
Species	6 month	6 mo or 1 yr	Rat	Mouse
Adrenals	X*	X*	X*	X*
Aorta	X	X	X (thoracic)	X (thoracic)
Bone Marrow smear	X (taken but not examined)	X (rib)		
Bone (femur)	X (incl marrow)	X (rib) (also sternum, but not processed)	X (with joint)	X (with joint)
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	X* (with uterus)	X	X (w/uterus)	X (w/uterus)
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X	X*	X*
Esophagus	X	X	X	X
Eye	X (w/optic nerves)	X (w/optic nerves)	X	X
Fallopian tube				
Gall bladder	N/A	X	N/A	X
Gross lesions		X	X	X
Harderian gland	X		X	X
Heart	X*	X*	X*	X*
Ileum	X	X	X	X
Injection site				
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland		X	X	X
Larynx			X	X
Liver	X*	X*	X*	X*
Lungs	X	X (w/ bronchi)	X*	X*
Lymph nodes, cervical	X	X		
Lymph nodes mandibular			X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X (inguinal)	X	X (caudal)	X (caudal)
Nasal cavity				
Optic nerves	X (w/eyes)	X (w/ eyes)	X	X
Ovaries	X*	X*	x* (w/oviducts)	X* (w/oviducts)
Pancreas	X	X	X	X
Parathyroid	X (w/thyroid)	X (w/thyroid)	X (w/thyroid)	X (w/thyroid)
Peripheral nerve				
Pharynx				

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Pituitary	X*	X*	X	X
Prostate	X*	X*	X	X
Rectum	X (not processed or examined)	X (not processed or examined)	X	X
Salivary gland	X	X (submandibular	X (submandibular)	X (submandibular)
Sciatic nerve	X	X	X	X
Seminal vesicles	X*	X	X	X
Skeletal muscle	X (biceps)	X (biceps femoris, Diaphragm [not processed])	X	X
Skin	X (dorsal thorax	X (dorsal thorax)	X	X
Spinal cord	X	X (cervical, lumbar, mid-thoracic)	X	X
Spleen	X*	X*	X*	X*
Sternum	X (w/bone marro		X	X
Stomach	X	X (cardia, fundus, pylorus)	X	X
Testes	X*	X*	X*	X*
Thymus	X	X	X*	X*
Thyroid	X* (w/ parathyroids)	X* (w/ /parathyroids)	X*	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X* (w/cervix)	X	X* (w/ cervix)	X* (w/cervix)
Vagina	X	X	X	X
Zymbal gland			X (w/external ear)	(not processed)

X, histopathology performed
*, organ weight obtained

Other Tissues examined:
6M Rat: pineal gland
6/12 M Monkey: pineal gland
Rat Carcinogenicity: femur, preputial gland, clitoral gland, ureters
Mouse Carcinogenicity: femur, preputial gland, clitoral gland, ureters

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/s/

MELISSA K BANKS-MUCKENFUSS
10/30/2013

LOIS M FREED
10/30/2013

PHARMACOLOGY/TOXICOLOGY
FILING CHECKLIST FOR NDA

NDA/BLA Number:205,677 **Applicant: Vanda Pharmaceuticals** **Stamp Date: 5/31/13**

Drug Name: tasimelteon **NDA Type: Priority**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Generally, the standard studies appear to have been conducted; however, a standard safety pharmacology battery was not conducted and a pivotal study (6-month rat; see taj0007-98348) lacks a separate, signed pathology report (as requested in the pre-NDA meeting minutes, dated 3/22/13). While the major human metabolites were not measured in the nonclinical studies, the sponsor conducted PK studies including the metabolites after oral administration in rats (29 days; parent, M3, M9, M12, M13, and M14 ; note that rat does not make M11), mice (29 days; parent and M11), pregnant rabbits (19 days; parent and M11), and monkeys (single dose only). There is a problem with the measurement of plasma M3 in rat.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		In most studies, tasimelteon was administered to animals by oral gavage (in PEG-400). No studies used the clinical formulation.

Reference ID: 3341349

PHARMACOLOGY/TOXICOLOGY
FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		This statement can be found in the Nonclinical Overview (page 4, last paragraph).
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		The sponsor included 2 tables specifying the identifiers used for tasimelteon main metabolites (cf. 2.6.4 Pharmacokinetics Written Summary, Table 1 pg. 10 and Table 20 pg. 53).
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The sponsor has provided labeling, the adequacy of which is a review issue. Toxicity study margins were given as "x times the human systemic exposures at the RHD" and were sometimes given only for effect levels (e.g., for the EFD studies). A no-effect level comparison was given for the fertility studies, the pre-/post-natal study and for tumors in the carcinogenicity studies.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Discussion with the CMC reviewer indicated that there are no impurity issues at this time.
11	Has the applicant addressed any abuse potential issues in the submission?			This is not Pharm/Tox purview; the CSS have been consulted.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			n/a

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE?
__Yes___

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter:

1) We request a separate, signed, and dated Pathology Report for the pivotal 6-month toxicity study in rats (Study TAJ0007-98348).

Reference ID: 3341349

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/s/

MELISSA K BANKS-MUCKENFUSS
07/15/2013

LOIS M FREED
07/15/2013